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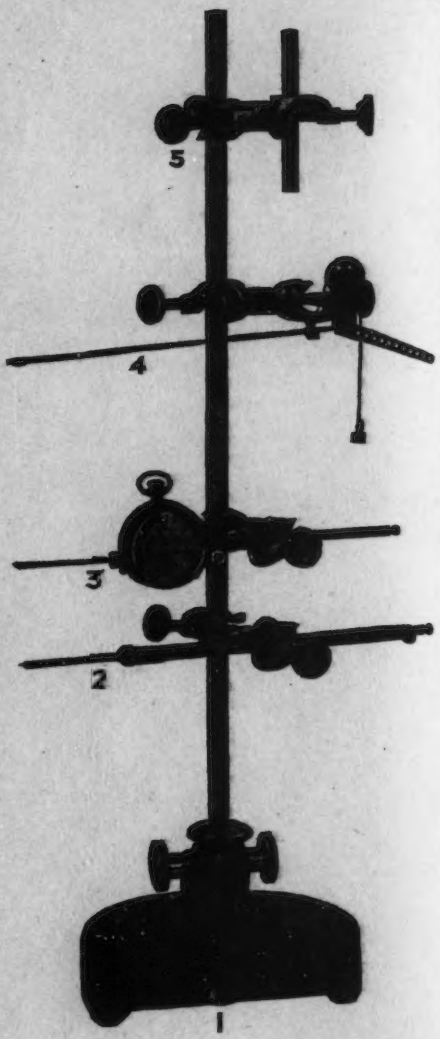

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THE EFFECTS OF LIGATION OF PANCREATIC DUCTS ON GASTRIC DIGESTION

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The process of digestion is an orderly one. Normally there appears to be perfect coördination between the mechanism propelling food through the alimentary tract and the pouring out of the proper digestive juices at the respective sites. Many factors are responsible for the coördination of the various processes necessary to prepare food for absorption. The most important of these are the nervous and chemical. The former has to do not only with the partial control of the motor mechanism but with initiating the secretion of the various digestive juices. Fundamentally the fact that some of the digestive juices are alkaline, while one of the most important is acid, suggests that an acid-alkaline mechanism may also be of importance. What part the various secretions that are poured into the intestinal tract have to do with maintaining this orderly sequence of the processes of digestion is not fully determined, although there is much work to substantiate the view that each has a significant part. On the other hand, it has also definitely been proved that the digestive processes are maintained without some of the digestive secretions. It is evident, therefore, that to a certain degree physiologic compensation can be made for gastric juice, bile, pancreatic secretion, duodenal secretion, and so forth, and the processes of digestion can be maintained, although impaired. The pancreatic juice is of great importance in the mechanism of digestion not only because three of the most valuable enzymes are contained in it but because of its alkaline character. It therefore seemed desirable to determine whether this orderly process of digestion would be impaired in a demonstrable manner by the loss of the pancreatic juice, and if so, how? Accordingly, a series of experiments was carried out in which the gastric digestion was studied both as regards the secretion of gastric juice and the

emptying of the stomach before and after the occlusion of the pancreatic ducts.

Various investigators studied the problem regarding the rate of discharge of food from the stomach, notably Beaumont, Leube, Penzoldt and Cannon. Cannon stated that the stomach is emptied in about three hours. Opinion is divided regarding the mechanism by which gastric acidity is reduced. The most recent views on the subject are those of Boldyreff who considers pancreatic juice as the chief alkalinizer, not only in the intestines adjacent to the stomach, but within the stomach itself. When pancreatic juice was not allowed to enter the stomach, neutralization of gastric juice went on tardily and the emptying of the stomach was carried on much more slowly than under normal conditions. Boldyreff maintained that the acid of the stomach, entering the intestines, irritates them, provoking antiperistalsis which drives the alkaline secretions of the intestines into the stomach, reducing the acidity.

METHOD OF RESEARCH. Five adult dogs, of medium size, to offset any difficulties in gastric lavage, were selected. The dogs were placed in individual kennels so as to keep the environmental conditions constant, and were given a standard test-meal, consisting of a mixture of 70 grams of ground up, raw, lean horse meat and 250 cc. of water. The meal was given on the morning of the experiment, after food had been withheld for from eighteen to twenty-four hours. To determine the normal gastro-acid curve, a lapse of half an hour was allowed for the food to be digested and then the contents were withdrawn by the fractional method, that is, from 10 to 15 cc. of the chyme, every half-hour until free hydrochloric acid appeared. After this, fractions were taken every fifteen minutes until the stomach was empty, as determined by manipulation of the stomach tube; when chyme did not appear at the end of repeated suctions, the stomach was inflated with a few cubic centimeters of air from an air-line attached to the same flask, and then the stomach tube was moved up and down until it moved freely in the stomach. This technic was suggested by McCann. The air was discontinued and suction reapplied to remove all the contents of the stomach. If chyme did not come through the tube after this procedure the stomach was considered empty of food and the experiment brought to a close. It was found that the dog's stomach was empty in from three to four hours. The individual samples (about 700 in all) were then centrifuged for from three to five minutes and gastric analysis was carried out.

Topfer's indicator was used for the determination of free hydrochloric acid, as suggested by Folin. Decinormal sodium hydroxide was used for the determination of total acids. The indicator was 1 per cent alcoholic solution of phenolphthalein. The Volhard-Harvey method was used for the determination of the chlorides. Five such experiments were carried out on each animal until a normal gastric-acid curve was established.

TABLE 1

Emptying time of stomach and gastric acidity before and after ligation of pancreatic ducts

DOG	WEIGHT	EMPTYING TIME OF STOMACH (ROENTGEN-RAY)		AVERAGE						MINIMUM						MAXIMUM					
				Chloride		Hydrochloric acid		Total acid		Chloride		Hydrochloric acid		Total acid		Chloride		Hydrochloric acid		Total acid	
		Before operation	After operation	Normal	Post-operative	Normal	Post-operative	Normal	Post-operative	Normal	Post-operative	Normal	Post-operative	Normal	Post-operative	Normal	Post-operative	Normal	Post-operative	Normal	Post-operative
	kgm.	*hours*	*hours*																		
1	8.7	4–5	3–4	1.3	1.2	20.4	15.2	60.6	73.8	0.1	0.4	8.0	2.0	28	24	2.9	2.2	44	25	118	122
2	6.4	4–5	3–4	1.1	1.3	17.6	21.0	48.4	61.0	0.3	0.4	5	10	20	32	3.0	2.8	34	40	90	115
3	7.3	3–4	3–4	1.0	1.2	0	19	45	63.6	0.1	0.4	0	5	10	24	2.5	2.8	0	30	110	100
4	8.2	3–4	2–3	1.5	1.6	16.5	18	59.1	71.3	0.3	0.2	6	10	14	30	2.5	2.6	40	40	120	120
5	8.7	4–5	3–4	1.8	1.7	24.2	17	73	75.0	0.5	0.6	5	8	20	31	2.8	2.9	60	61	126	128

To determine the normal emptying time of the stomach by means of roentgen rays, a meal was given consisting of 70 grams barium sulphate, 70 grams solution of acacia, and 70 grams condensed (evaporated) milk. The first examination was made when the stomach was full and then the rate of discharge of the meal from the stomach into the duodenum was noted by means of the fluoroscope. Other examinations were made two, three and four hours after the meal. The normal emptying time of the stomach of each dog was thus determined and found to vary from three to five hours (table 1).

Under deep ether anesthesia and aseptic technic, an incision from 6 to 8 cm. long was made in the wall of the abdomen through the linea alba, beginning at the ensiform cartilage. The duodenum was then brought to the surface and laid toward the right side so as to expose the site of the

TABLE 2

Protocol of a representative experiment

NORMAL				AFTER LIGATION			
Time	Chlorides	Free HCl	Total acid	Time	Chlorides	Free HCl	Total acid
a.m.				*p.m.*			
9:00	0.4	0	42	2:00	0.6	0	24
9:30	0.8	0	72	2:30	0.8	0	46
10:00	1.2	0	110	3:00	1.1	0	74
10:15	2.0	0	110	3:30	1.6	Trace	100
10:30	2.6	0	90	3:45	1.2	0	50
10:45	2.5	12	80	4:00	Stomach empty		
11:00	1.5	10	60				
11:15	1.3	0	40				
11:30	Stomach empty						

pancreatic ducts. Following Mann's technic, an aneurysm needle was passed beneath the major and minor ducts; the ducts were then ligated with silk on the intestinal side and evulsed along the entire course in the pancreas on the pancreatic side. To insure complete discontinuity of all of the pancreatic ducts, the pancreas was separated from its attachment to the duodenum in two dogs by means of gauze dissection and the major and minor ducts ligated and evulsed at the same time. The abdomen was then closed in the usual manner. Recovery was uninterrupted and the animals regained full strength by the eighth day after operation for the fractional gastric analysis. The essential change noted in the stools following the operation was that they were semisolid and clay-colored. Diarrhea or vomiting did not occur.

On the ninth day after the operation, the dogs were again given the

test-meal, and fractional analysis was carried out at fifteen-minute intervals, as before operation, until the stomach was completely empty. Post-operative gastric acid curves were constructed and the emptying time of the stomach studied by means of roentgen rays, as before operation.

RESULTS. In the entire series, the acid curves remained practically the same after operation as before complete ligation of the pancreatic ducts. On the other hand the stomach emptied faster and was usually empty one hour sooner after operation than before (table 2).

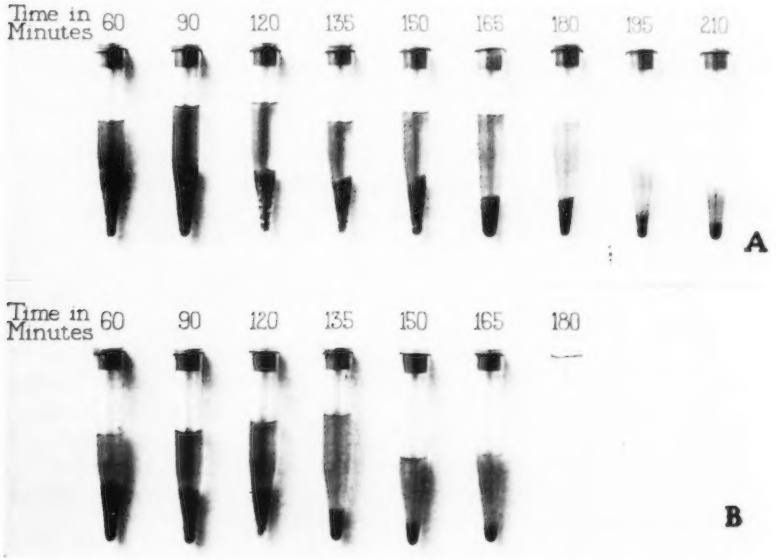

Fig. 1. Fractions of gastric contents withdrawn at intervals before and after operation. The meal consisted of 70 grams lean ground up horse meat and 250 cc. tap water. *A*, normal animal. *B*, following evulsion of pancreatic ducts.

It was incidentally observed that the meat given in the tests after operation was digested faster than by the normal animal. A finer residue of meat was obtained in every tube for the respective time in the post-operative tests as compared to the preoperative tests (fig. 1). In order to determine whether any chemical change occurred in the blood after ligation of the pancreatic ducts, the blood amylase was studied by the Elman and McCaughan method and a definite increase was found in every animal.

The dogs appeared to be normal; after operation they ate as rapidly as before operation, but as would be anticipated, gradually lost weight.

An attempt was made to maintain weight by feeding 50 grams of whole beef pancreas daily, with milk and syrup. The success of the evulsion of the pancreatic ducts was verified at postmortem examination: the pancreas had almost completely atrophied.

Discussion. In the experiments following ligation and occlusion of pancreatic ducts in dogs, several interesting points developed. First, it was noted that there was a decrease in the emptying time of the stomach of the dogs thus operated on. This was persistently observed in the fluoroscopic examination and roentgenograms made hourly after the food was taken. The decreased emptying time was also noted in the fractional analysis tests. The stomach of the normal dog emptied completely at approximately the end of three hours, and after operation it emptied the same quantity of food completely at approximately the end of two hours. Such results were obtained in each dog.

The second point observed was the acceleration or speeding up of intragastric digestion. Each sample withdrawn from the stomach revealed a marked degree of digestion as compared with preoperative samples of the same kind and quantity of food. Just how these changes are brought about is difficult to explain satisfactorily. One might assume that it is due to the absence of any reflex alkaline pancreatic juice emptying into the stomach or possibly to the fact that when the pancreatic digestive mechanism is no longer functioning the gastric mechanism is stimulated to greater activity. Striking changes were not observed in gastric acidity after complete ligation of pancreatic ducts. Total acids, free hydrochloric acid, and chlorides were carefully studied in each sample collected, and averages were noted of the data from the normal animals and from animals after operation. Striking changes (fig. 1) did not occur after ligation of the ducts, as compared with those before operation.

SUMMARY

The study of the effect of ligation and evulsion of pancreatic ducts revealed that: 1, in the absence of all the pancreatic secretion there is only a slight rise in gastric acidity; 2, the stomach empties faster after ligation of the pancreatic ducts; 3, there is increased protein digestion in the stomach and increase in blood amylase following evulsion, and 4, good health is maintained but loss of weight is progressive when both pancreatic ducts are ligated and evulsed.

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CARBON DIOXIDE FROM THE NERVES OF COLD-BLOODED VERTEBRATES

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Several years ago I attempted to determine by means of a modification of Osterhout's colorimetric apparatus the rate of production of carbon dioxide by nerves from cold-blooded vertebrates. Since that time several important contributions to this subject have been made, notably by Gerard and by Fenn, whose results I wish to discuss in connection with certain new observations of my own.

In my earlier work the calibrating of the respiratory chamber in my apparatus presented serious difficulties, but a method to overcome the obstacles was suggested to me by Dr. E. J. Cohn, who at the same time advised its application to a similar problem in the hands of Dr. G. B. Ray. In the absence of Doctor Cohn I followed the procedure employed by Ray (1924) and published my results. On looking over what I had printed Doctor Cohn kindly pointed out to me an inaccuracy into which both Ray and I had fallen and indicated the method of correction. This method was applied to the quantitative determinations in my paper by Davis (1926, p. 559) who showed that it was necessary to multiply my results by a correction factor of from 0.619 to 0.623 depending upon temperature. At about this time outside duties obliged me to be absent from Cambridge for almost a year, but on my return I undertook at once the recalibration of my apparatus with results that I now believe to be approximately final. In this work I was greatly aided by Doctor Cohn and Doctor Davis, to both of whom I wish to express my sincere thanks. I also wish to apologize to Doctor Cohn for having attributed to him in my earlier paper an inaccurate method for which he was not responsible.

The capacity of the respiratory chamber that was used in my latest work was 42 cc., and, following the method that was employed in my first paper to estimate the weight of carbon dioxide needed to change this chamber from pH 7.78 to pH 7.36, I found the amount to be 0.027,72 mgm. (Parker, 1925a, p. 650). Adopting Doctor Cohn's correction and calculating the carbon dioxide separately for the 5 cc. of fluid and the 37 cc. of gas in the respiratory chamber at 23°C., the total amount proved to be 0.018,18 mgm. or a little less than 66 per cent of the estimate by the earlier procedure.

This percentage is very close to the correction factor, 0.623, used by Davis for my earlier results and differs from it because of the difference in the proportions of the liquid and the gas phases in this chamber as compared with that in the first chamber used.

Having arrived at this tentative determination for the new chamber, I deemed it desirable, notwithstanding my past difficulties, to devise a direct calibration for this part of the apparatus. To accomplish such a calibration it was necessary to measure accurately very minute amounts of carbon dioxide, and to deliver such measured amounts with certainty in the chamber. After trying numerous methods without great success, I adopted the following procedure.

As a means of measuring carbon dioxide I used a narrow-stemmed, high quality, mercury thermometer from which the end opposite the bulb had been cut off squarely, thus leaving the fine bore of the thermometer open to the air. On warming or cooling the bulb the mercury in the tube could be driven up or down with great regularity and precision, and any gas about the open end could thereby be sucked into the tube or driven out of it. By attaching a small millimeter scale to the tube the length of a contained column of gas could be easily read at any time. The scale used in these measurements was a narrow Keuffel and Esser celluloid scale divided into half-millimeters. If it was desired to close off the column of gas in the thermometer tube this could be done by dipping the open end of the tube during suction into mercury, a small amount of which would then be drawn into the tube and would thus form an effective seal. Columns of gas could in this way be isolated and measured at atmospheric pressure and their length determined easily and accurately to within half a millimeter. By calibrating the thermometer tube used in these tests it was ascertained that half a millimeter of length, the smallest unit used, represented 0.000,025 cc. or, at 23°C., 0.000,046 mgm. of carbon dioxide. Hence this method afforded a reasonably safe means of measuring the carbon dioxide called for in these tests where the smallest differences to be distinguished were of the order of 0.0003 mgm.

The gas employed in this work was manufactured by the Liquid Carbonic Corporation and delivered to the laboratory in heavy iron cylinders. Tests of the purity of this gas were kindly made for me in the laboratory of Prof. G. P. Baxter, and samples were shown to contain 99.8 per cent carbon dioxide. The slight difference between this and absolutely pure carbon dioxide was ignored in my calculations.

To fill the thermometer tube with a given volume of carbon dioxide the bulb of the thermometer was warmed with a small alcohol flame till the mercury reached the open end of the tube whereupon the tube with its attached scale was quickly introduced several centimeters into the mouth of a large glass delivery tube from which was flowing a generous current of

carbon dioxide. When the mercury in the bulb began to cool and that in the tube to retreat, carbon dioxide was thereby sucked into the thermometer tube and when this column of gas had reached the required length, as indicated by the attached scale, the open end of the tube was plunged into a small pool of mercury held in a slight enlargement at a bend in the general delivery tube. In consequence of continual suction a minute amount of mercury was thus drawn into the thermometer tube and the carbon dioxide thereby sealed off. After the gas and mercury had come to rest, the thermometer with its measured volume of carbon dioxide effectively sealed was removed from the delivery tube to the air.

As a preliminary step to discharging a measured amount of carbon dioxide into the respiratory chamber, it was necessary to set this chamber at a known pH value. This was done by inserting into the chamber through the small aperture in its stopper a capillary tube by which air free from carbon dioxide could be bubbled in considerable amounts through the indicator solution. This solution was thus reduced in acidity till its tint agreed with that of the outside standard tube for pH 7.78. The capillary tube was then withdrawn and the small aperture in the stopper was plugged with the mixture of kaolin and vaseline already described (Parker, 1925a, p. 649). The thermometer tube was now charged with a given volume of carbon dioxide, the plug of kaolin removed from the small stoppered aperture, and the free end of the thermometer tube inserted through this aperture for a distance of about five centimeters into the chamber. The thermometer bulb was immediately heated, whereupon the column of mercury and gas in the tube began to move toward the tube's open end. The sealing droplet of mercury was first forced out and clung to one side of the capillary opening on the glass face at the end of the tube. Then followed the discharge of all the carbon dioxide contained in the tube. When the mercury in the lower part of the tube had forced out all the contained carbon dioxide, the tube was carefully and quickly withdrawn from the chamber and the small aperture through which the tube had been inserted was again closed with the kaolin mixture. The whole operation was carried out in a well ventilated room, and as the discharge of the gas took place near the deep end of the chamber and the period during which the small aperture in the stopper was open was not over half a minute, it is assumed that the procedure was sufficiently guarded to have been successful. This assumption was justified by the uniformity of the resulting determinations.

A measured volume of carbon dioxide having thus been introduced into the respiratory chamber, the apparatus was then agitated for fifteen minutes in the usual way (Parker, 1925a, p. 645) to bring the mixture of contained gases and fluids to approximate equilibrium. The color of the indicator solution in the chamber was then compared with that of the

standard tube for pH 7.36 to ascertain whether the amount of introduced carbon dioxide had been too great, too small, or just sufficient to bring the chamber to this point. It was quickly discovered that 12 cm. of carbon dioxide was considerably too much for this purpose and that 3 cm. of this gas was too little. After numerous trials the requisite amount was found to be very close to 7.5 cm., 7.25 cm. being apparently always too little and 7.75 cm. too much. The tests were carried out at 23°C., and at this temperature one centimeter of tube length was estimated to contain 0.000,915 mgm. of carbon dioxide; consequently 7.5 cm. would be represented by 0.006,86 mgm. of this gas, the approximate weight necessary to change the chamber from pH 7.78 to pH 7.36.

When this weight of carbon dioxide is compared with those of the two earlier determinations, it is found to be under both. It is almost exactly 25 per cent of my erroneous reckoning of 0.027,72 mgm., and about 37 per cent of 0.018,18 mgm., the corrected theoretic reckoning. Why there should be a discrepancy between the theoretic amount and the amount actually found is difficult to say. It has been suggested that the indicator itself, the phenolsulphonephthalin, may have added to the acidity of the solution sufficiently to make itself felt when the sodium hydrogen carbonate was used at a dilution so great as 0.0001 mol. To test this suggestion solutions were made up in which the sodium hydrogen carbonate was at a concentration of 0.001 mol. and of 0.002 mol. and to each of these only the usual two drops of a saturated aqueous solution of phenosulphonephthalin were added per 10 cc. of solution. In working with these mixtures a more capacious thermometer tube was used in place of the smaller one first employed. When the indicator mixture was based on 0.001 mol. sodium hydrogen carbonate solution it was found that to change the chamber from pH 7.78 to pH 7.36 10 cm. of carbon dioxide from the new thermometer tube was clearly too much, and 9 cm. was too little, but that 9.5 cm. was very close to the requisite volume. The new tube proved to contain 0.0073 mgm. of carbon dioxide per centimeter; consequently in 9.5 cm. there must have been 0.0694 mgm. of this gas. This amount is almost exactly ten times the former determination of 0.00686 mgm., and shows that the same discrepancy occurs at 0.001 mol. as was found at 0.0001 mol. When the indicator solution was based on a 0.002 mol. solution of sodium hydrogen carbonate, a volume of carbon dioxide twenty times that needed in the original test or 0.1372 mgm. was introduced and was found just sufficient to change the chamber as shown by the color of the indicator from pH 7.78 to pH 7.36. These two results make clear that the disagreement between the theoretic and the actual determinations is not dependent upon the acidity introduced by the phenolsulphonephthalin, for the amount of carbon dioxide needed to make the necessary change with these higher concentrations is still at about 37

per cent of the theoretic amount. What the occasion of this discrepancy can be is difficult to say. Doctor Cohn and Doctor Davis have both been good enough to go over my apparatus and procedure with care, and are at a loss to suggest an explanation. Apparently the present application of the indicator method points to a chemical situation not yet clearly understood. Notwithstanding this difficulty, I believe from the way in which the direct calibration of my apparatus has been made that I am justified in accepting 0.006,86 mgm. as the weight of carbon dioxide necessary to change my present respiratory chamber, under the conditions in

TABLE 1

Weights of carbon dioxide discharged by isolated vertebrate nerve, expressed in milligrams per gram of whole nerve per minute

INVESTIGATOR	YEAR	SPECIES	TEMPERATURE	CO_2 IN MGM. PER GM. OF NERVE PER MINUTE		
				Minimum	Maximum	Average
Rabbit						
			°C.			
Thunberg	1904		16	0.00068	0.00089	
Frog						
Tashiro	1913	R. pipiens	19–20			0.0055
Riggs	1919	R. sp.?	20			0.0025
Parker	1925	R. grylio	22	0.0018	0.0028	0.0022
Gerard	1927	R. esculenta	14			0.00038
Gerard	1927	R. temporaria	14			0.00051
Fenn	1928	R. pipiens?	22?	0.0024	0.0031	0.0027
Parker	1928	R. clamitans	22	0.0026	0.0037	0.0032
Parker	1928	R. grylio	22	0.0031	0.0040	0.0034
Dogfish						
Parker	1925		22	0.0018	0.0032	0.0024
Fenn	1927		22	0.0015	0.0030	0.0020

which I have worked, from pH 7.78 to pH 7.36, and I shall therefore use this determination in my subsequent discussion.

CARBON DIOXIDE FROM NERVE. *1. General.* The more important determinations of the amounts of carbon dioxide given out by excised quiescent nerves are briefly summarized in table 1. Notwithstanding the obvious advantage of cold-blooded nerves for this purpose, the first determinations of this kind were made by Thunberg (1904) on the nerves of a warm-blooded animal, the rabbit. The large leg nerves of this animal were excised and cooled to 16°C. They yielded in a microrespirometer, as the table shows, only a small proportion, about a third or a fourth, of the

carbon dioxide obtained by most subsequent workers on the leg nerves of the frog. This deficiency is probably due in part at least to the low temperature at which the tests were made, but also to the thickness of the nerves, a feature which, as Fenn (1927a, p. 342) and others have pointed out, prevents the complete escape of much of the carbon dioxide produced in the core of the nerve.

2. Frog nerve. The determinations of carbon dioxide made in the past by workers on frog nerves show considerable range. For these measurements Tashiro (1913) and Riggs (1919) used the biometer, an apparatus whose quantitative accuracy has been called in question (Adam, 1921; Parker, 1925a). Hence it is not surprising to find that Tashiro's determination is more than twice that of Riggs. Riggs' results are, however, very close to those of several later workers, but as the biometer method is probably qualitative rather than quantitative I am inclined to regard this agreement as accidental and not representative of a reliable measurement. The determinations of carbon dioxide from frog nerves by Fenn (1928, p. 116) and by me (Parker, 1925b) are in fair agreement. Collectively they diverge somewhat from those of Gerard (1927c, p. 386), which are only about a fifth to an eighth of ours. That Gerard's determinations were made at a temperature of 14°C. in contrast with 22°C. is a partial explanation of this difference, but it is quite improbable that this is the whole explanation.

Two factors that are of significance in these determinations and that are only now beginning to be appreciated, are the diameters of the nerves tested and the seasons of the year at which the tests are made. As already stated, Fenn (1927a, p. 342) has pointed out that in thick nerves much of the carbon dioxide produced near the core of the nerve is probably too deep-seated to escape by diffusion and consequently must accumulate there to the detriment of that part of the nerve. This is probably the chief occasion for the low rate of carbon dioxide production in Rana grylio (Parker, 1925b), where the nerves are relatively thick, as compared with the rate in the species tested by Fenn (1928) where the nerves are much smaller in diameter. That the observed difference in the rates of these two nerves is not due to inherent differences in the methods used by Fenn and by me is seen from the fact that when I tested in my own apparatus a set of nerves from Rana clamitans of about the same weight as those used by Fenn (1928, p. 116) I reached practically the same results as Fenn did (compare table 1). The diameter of an excised nerve is certainly a significant element in dealing with its rate of carbon dioxide production.

Although Fenn (1927a, p. 776) was unable to discover any seasonal variation in the output of carbon dioxide from the frog nerves he worked upon, Gerard (1927c, p. 386) noted that nerves from spring frogs gave out carbon dioxide at considerably higher rates than those from winter frogs.

The increases observed amounted to from 22 to 31 per cent of the ordinary winter rates. My own results from Rana grylio support Gerard's view. During the winter this species is very sluggish and quiet, and under such circumstances I found the rate of carbon dioxide production to average 0.0022 mgm. of gas per gram of nerve per minute (Parker, 1925b). In the early summer Rana grylio becomes extremely active and nerve preparations made from it at this time of year showed an average rate of 0.0034 mgm. of gas per gram of nerve per minute, an increase of about 55 per cent over the winter rate. I am fully persuaded, therefore, that the season of year is an important factor in the rate of carbon dioxide production in cold-blooded nerve. The exceptionally low records obtained by Gerard (1927c) may have been due in part at least to such seasonal conditions, but to what extent this is so remains to be determined. From Fenn's records and my own it seems clear that in species of small frogs during the winter quiescence the rate of carbon dioxide production may vary from 0.0024 mgm. to 0.0037 mgm. of gas per gram of nerve per minute.

3. Lateral-line nerve of dogfish. The lateral-line nerves of the common smooth dogfish were studied at Woods Hole by Fenn (1927a) and by me (Parker, 1925a). As table 1 shows the two sets of determinations are in close agreement and it is fair to conclude from them that carbon dioxide is discharged by this nerve at a rate of from 0.0020 mgm. to 0.0024 mgm. of gas per gram of nerve per minute, rates that are in general a little lower than those already recorded for the frog.

The fact that the lateral-line nerve is a purely sensory nerve of great uniformity of structure makes it an unusually favorable object for work on several important questions. One of these is the rate of carbon dioxide production of different parts of a nerve. Tashiro (1914) some time ago declared that the proximal part of the claw nerve (mostly efferent) of the spider crab discharged carbon dioxide at about twice the rate of the distal part and that in general the reverse was true of the optic nerve (afferent) of Limulus. He subsequently published (Tashiro, 1917, p. 78) determinations for the carbon dioxide output of the lateral-line nerve of the carp to the effect that the distal portion of this nerve discharged carbon dioxide at a rate about three times that of the proximal portion. These observations led to the view that nerves exhibit metabolic gradients which diminish peripherally in motor fibers and centrally in sensory fibers; in other words, such gradients diminish in the direction in which the nerve impulse naturally flows. These views were incorporated and elaborated by Child (1921) in his general theory of metabolic gradients. Gerard (1927c, p. 387), working on the sciatic nerve of the frog, found that the upper and lower halves of this nerve were indistinguishable in metabolic rate, but he rightly concluded that in consequence of the mixed nature of this nerve his findings were not inconsistent with the presence of gradients on individual fibers.

In the lateral-line nerve of the dogfish all fibers are sensory and consequently if a metabolic gradient is present it should be easily demonstrable. To test this possibility five lateral-line nerves of the dogfish were removed from the animals and were cut into approximately distal and proximal halves. Beginning about two hours after their removal, when the period of gush could be expected to be over, the rate of carbon dioxide outflow was determined for each of the two parts of these nerves. The results of these determinations are given in table 2.

The determinations recorded in this table show that in two instances the proximal parts of the nerves gave slightly higher rates of carbon dioxide production than the distal parts, and that in three the distal parts were a little higher than the proximal parts. In all instances the differences are relatively slight. From the distribution of these differences, in some instances in favor of the distal parts and in others in favor of the proximal parts, and from the close agreement of the averages, I conclude that the metabolic rates in the two parts of this nerve are not significantly

TABLE 2

Rates of carbon dioxide production in milligrams of carbon dioxide per gram of nerve per minute from the distal and the proximal parts of the lateral-line nerve of the dogfish

PARTS OF NERVE	NUMBER OF NERVE					AVERAGES
	1	2	3	4	5	
Proximal	0.0026	0.0031	0.0024	0.0019	0.0022	0.00244
Distal	0.0023	0.0028	0.0026	0.0027	0.0025	0.00258

different. Certainly there is no evidence in favor of Tashiro's view that the distal part of the lateral-line nerve has a metabolic rate some three times that of the proximal part. I therefore cannot agree with those (Child, 1921; Herrick, 1924) who accept the view that there is a metabolic gradient in nerve.

In a former paper (Parker, 1925c) I tabulated a series of determinations to show the weight of carbon dioxide discharged in one minute by one centimeter of nerve fiber in the excised quiescent lateral-line nerve of the dogfish. Subjecting these determinations to the necessary correction mentioned in the present paper, I recalculated the results for each of the two nerves tested as shown in table 3.

Leakage having been deducted the remaining weights of carbon dioxide, called in the table "organic," are believed to be the actual amounts of this gas discharged by the two nerves. On the basis of these determinations it can be shown that the rates of discharge in milligrams of carbon dioxide per gram of nerve per minute are for nerve I 0.0023 and for nerve II 0.0026, amounts very close to the averages already recorded in table 1. But this

carbon dioxide comes from the whole nerve, from its capillaries, connective tissue, and other such parts, as well as from its nerve fibers. By methods already described (Parker, 1925c, p. 192) it was possible to divide the "organic" carbon dioxide of table 3 between the non-nervous and the nervous components of the two nerves. The nervous component of nerve I was 60 per cent of its weight or 76.8 mgm. and of nerve II 53 per cent or 74.2 mgm. These are the weights of tissue that are responsible for the production of the "nervous" carbon dioxide given in table 3. From these several determinations a rate of discharge can be calculated which will apply to the strictly nervous components of the two nerves. Carrying out this calculation it appears that in nerve I this rate of discharge is 0.0033 mgm. of carbon dioxide per gram of nerve fibers per minute and

TABLE 3

Carbon dioxide in milligrams discharged by two lateral-line nerves, I and II, from the smooth dogfish, Mustelus canis (Mitchell)

From the total carbon dioxide is subtracted in the case of each nerve the leakage carbon dioxide and the non-nervous carbon dioxide. The resulting nervous carbon dioxide is then divided by the average number of minutes for the tests, the lengths of the nerves in centimeters, and finally by the average numbers of nerve fibers in each nerve. The weight of nerve I was 128 mgm., of nerve II, 140 mgm.

	NERVE I	NERVE II
Total CO_2	0.004,800	
Leakage	0.000,352	0.000,298
Organic CO_2	0.004,448	0.004,502
Non-nervous CO_2	0.000,720	0.000,781
Nervous CO_2	0.003,728	0.003,721
For 1 minute	0.000,252	0.000,298
For 1 cm	0.000,021	0.000,019,6
For 1 fiber	0.000,000,011	0.000,000,011

in nerve II 0.0040 mgm. As these rates of discharge are based upon determinations that apply strictly to nerve fibers and not to other associated tissues, they represent a more accurate form of statement as to the rate of discharge of carbon dioxide by nervous tissue than those hitherto given.

If we assume that the average rate of discharge of carbon dioxide from the whole lateral-line nerve of the dogfish is 0.0022 mgm. per gram of nerve per minute and that the average rate of discharge for the nerve fibers in such a nerve is 0.00365 mgm. per gram of nerve fibers per minute then the nerve-fiber rate exceeds the general rate by about 66 per cent of the latter.

In the two lateral-line nerves upon which table 3 is based the number of nerve fibers were found to average for nerve I 1911.5 and for nerve II

1841 (Parker, 1925c, p. 194). Dividing the amount of "nervous" carbon dioxide discharged from these nerves (table 3) by the average number of minutes over which the tests extended, by the length of the nerves in centimeters, and by the numbers of fibers just given, it can be shown that the amount of carbon dioxide discharged by 1 cm. of lateral-line nerve fiber per minute is 1.1×10^{-8} mgm. (table 3).

The increase in carbon dioxide output on stimulating the lateral-line nerve of the dogfish was found by me to be 15.8 per cent (Parker, 1925a) and by Fenn (1927a, p. 345) to be 15 per cent. These determinations are so close that it seems fair to conclude that the increase is not far from 15.4 per cent and that consequently the extra carbon dioxide discharged by one

TABLE 4

Carbon dioxide in milligrams per gram of nerve per minute and corresponding calories from resting and from stimulated nerve

The nerves used were lateral-line nerves of the dogfish and sciatic nerves of the frog. In the table a number preceded by an asterisk is a number obtained by calculation; one not so designated is a direct determination.

INVESTIGATOR	YEAR	ANIMAL	TEMPERATURE	CO_2	CALORIES
Resting nerve					
			°C.	mgm.	
Parker	1925	Dogfish	22	2.6×10^{-3}	$*6.6 \times 10^{-3}$
Fenn	1927	Dogfish	22	2.0×10^{-3}	$*5.1 \times 10^{-3}$
Gerard	1927	Frog	14	$*0.5 \times 10^{-3}$	1.1×10^{-3}
Fenn	1928	Frog	22?	2.7×10^{-3}	$*7.9 \times 10^{-3}$
Parker	1928	Frog	22	3.2×10^{-3}	$*8.1 \times 10^{-3}$
Excess on stimulating nerve					
Parker	1925	Dogfish	22	4.1×10^{-4}	$*10.4 \times 10^{-4}$
Fenn	1927	Dogfish	22	2.9×10^{-4}	$*7.5 \times 10^{-4}$
Parker	1928	Frog	22	4.5×10^{-4}	$*11.4 \times 10^{-4}$

centimeter of stimulated nerve fiber from the lateral-line nerve of the dogfish would amount to 0.154 times 1.1×10^{-8} mgm. or 1.7×10^{-9} mgm. (table 5).

The nerves used in these tests were stimulated by means of a Harvard inductorium which yielded very close to one hundred stimuli per second. If the excess carbon dioxide due to stimulation, 17×10^{-9} mgm. per minute, is now divided by the approximate number of stimuli per minute, namely 6000, the resulting amount, 2.8×10^{-13} mgm., is the weight of carbon dioxide discharged by one of a series of nerve impulses in passing over one centimeter of nerve fiber from the lateral-line nerve of the dogfish (table 6).

DISCUSSION. The results of recent work on the absorption of oxygen by nerve (Haberlandt, 1911; Adam, 1921) and particularly on the discharge of carbon dioxide by that tissue (Tashiro, 1913; Moore, 1919; Parker, 1925a) led Hill and his co-workers to renew the search for heat as the result of nerve stimulation. It was found by these investigators (Downing, Gerard and Hill, 1926; Hill, 1927; Gerard, 1927a, 1927b;

TABLE 5

Carbon dioxide in milligrams per minute and corresponding calories from a resting and from a stimulated single nerve fiber one centimeter long

In some instances the calculations were based on the carbon dioxide given out by the *whole nerve* ("organic") and in others on that given out by the *nerves fibers* only ("nervous"). The asterisk is used as in table 4.

INVESTIGATOR	YEAR	BASIS	ANIMAL	TEMPERATURE	CO_2	CALORIES
Resting nerve fiber						
				°C.		
Parker	1928	Organic	Dogfish	22	1.3×10^{-8}	$*3.3 \times 10^{-8}$
Parker	1928	Nervous	Dogfish	22	1.1×10^{-8}	$*2.8 \times 10^{-8}$
Excess on stimulating nerve fibers						
D. G. H.	1926	Organic	Frog	15	$*1.7 \times 10^{-9}$	4.2×10^{-9}
Parker	1928	Organic	Dogfish	22	2.1×10^{-9}	$*5.3 \times 10^{-9}$
Parker	1928	Nervous	Dogfish	22	1.7×10^{-9}	$*4.3 \times 10^{-9}$

TABLE 6

Carbon dioxide in milligrams and corresponding calories discharged by the passage of a single nerve impulse over a single nerve fiber one centimeter in length

The asterisk is used as in table 4

INVESTIGATOR	YEAR	BASIS	ANIMAL	TEMPERATURE	CO_2	CALORIES
				°C.		
D. G. H.	1926	Organic	Frog	15	$*1.0 \times 10^{-13}$	2.5×10^{-13}
Gerard†	1927	Organic	Frog			10.0×10^{-13}
Parker	1928	Organic	Dogfish	22	3.5×10^{-13}	$*8.9 \times 10^{-13}$
Parker	1928	Nervous	Dogfish	22	2.8×10^{-13}	$*7.1 \times 10^{-13}$

† Rough estimate only.

Gerard, Hill and Zotterman, 1927) that not only was a measurable amount of heat produced, but that it appeared in two phases, initial heat and retarded heat, the first representing in all probability the passage of the impulse and the second a process of recovery. Meanwhile the gas exchange of nerve had been extensively investigated (Fenn, 1926, 1927c, 1927d; Gerard, 1927b, 1927c) with the result that a chemical picture of

nerve activity was beginning to appear (Gerard and Meyerhof, 1927; Gerard, 1927d). Most of this work as a matter of convenience was done on frog nerves but such nerves are structurally complex and it is greatly to be regretted that, for instance, no heat determinations have as yet been made on the relatively simple lateral-line nerves of such creatures as the dogfish.

As a basis of comparison for these various results table 4 has been compiled. In this table is shown in milligrams of carbon dioxide produced by one gram of nerve per minute as well as in calories the activity of resting and of stimulated nerve. In some instances the original records were stated in weights of carbon dioxide, and from these records calories have been calculated on the assumption that the heat was the result of the oxidation of glucose. In other instances the original records were in calories, in which cases the carbon dioxide has been calculated from the heat. Naturally the table gives only a rough basis for comparison.

An inspection of table 4 brings out several important points. The difference between Gerard's determinations and those by Fenn and by me for resting nerve has already been discussed and need not be referred to again. Comparisons of Fenn's determinations and mine show that the rates in dogfish nerves are somewhat lower than those in frog nerves, a not unnatural condition. It is also evident that Fenn's measurements both on dogfish and on frog material are consistently slightly lower than mine, a condition probably dependent upon the individual peculiarities of our methods. This difference also reappears in the part of the table showing the results of stimulation. Here too dogfish nerve stands lower than frog nerve.

In table 5 are given weights of carbon dioxide as well as calories per centimeter of nerve fiber per minute. In this table two bases of calculation have been employed. In one the carbon dioxide from the whole nerve (called "organic" in table 3) has been apportioned among the nerve fibers. In the other, the more accurate, only such carbon dioxide (called "nervous" in table 3) as was discharged by the nerve fibers themselves has been apportioned to them. Naturally the amounts of carbon dioxide on the first plan are always larger than those on the second and this is shown in both parts of the table. In calculations carried out by Downing, Gerard and Hill (1926) what has been here called the second plan was used. The result that they arrived at when transposed from calories into milligrams of carbon dioxide, namely, 1.7×10^{-9}, agrees very closely with my direct determination, 2.1×10^{-9} mgm. This agreement, however, may be somewhat deceptive. In the first place the number of nerve fibers assumed by Downing, Gerard and Hill to be in the sciatic nerve of the frog was taken by them from the work of Dunn (1900, 1902, 1909), which was carried out at a time when medullated fibers were believed to be the only

type of fiber in such nerves. Since then it has been demonstrated that nerves like the sciatic contain many non-medullated fibers. These additional fibers, if included in the calculation, would decrease the weight of carbon dioxide attributable to each fiber and consequently should yield a result smaller than that recorded in the table. The fact, however, that Downing, Gerard, and Hill worked at 15°C. and that my work was done at 22°C. might tend, if this temperature relation could be introduced as a correction, to bring the two determinations together, though in any such adjustment it must be kept in mind that frog nerve regularly has a slightly higher rate of carbon dioxide production than dogfish nerve has. But quite aside from these minor differences, it must be perfectly evident that the two sets of determinations are so close as to be essentially of the same order of magnitude. It is therefore highly probable that the numbers given in table 5 represent with a fair degree of accuracy the carbon dioxide from one centimeter of quiescent nerve fiber and the additional weight discharged by this length of nerve fiber under stimulation.

Finally, in table 6 are set down for comparison carbon dioxide in milligrams and the number of calories given out when a single nerve impulse passes over 1 cm. of nerve fiber. Two independent determinations are shown, namely one by Downing, Gerard and Hill from the frog, 1.0×10^{-13} mgm., and the other by me from the dogfish, 3.5×10^{-13} mgm. Although these determinations are not in actual agreement, they are nevertheless so close that they may be assumed to represent, approximately at least, the weight of carbon dioxide or the number of calories released on the passage of one of a series of impulses over one centimeter of nerve fiber.

The nervous activity that is thus represented takes place in a single nerve fiber one centimeter long. An average nerve fiber from the lateral-line nerve of the dogfish has been found to have a diameter of 0.0116 mm. Its circular cut end would therefore have an area of almost exactly 0.0001 sq. mm. With a length of one centimeter the volume of such a nerve fiber would be very close to 0.001 cu. mm. From this volume of nervous substance at rest are given out per minute 1.1×10^{-8} mgm. of carbon dioxide. On stimulation this amount is increased by 1.7×10^{-9} mgm. of gas and when the nervous impulses pass over it at the rate of about one hundred per second each impulse is accompanied by a discharge of approximately 2.8×10^{-12} mgm. of carbon dioxide. If Avogadro's number is applied, it can be shown that this weight of carbon dioxide is composed of about 3,850,000 molecules, a number relatively so small as to suggest that the transmitting activity of a nerve fiber is limited to a very small portion of its substance such, for instance, as a surface layer, as implied in Lillie's (1923) conception of the operation, or to some other restricted portion.

SUMMARY

1. A method is described for the measurement of very minute volumes of carbon dioxide, down to 2.5×10^{-5} cc.

2. The rates of carbon dioxide production by the leg nerves of Rana grylio is in milligram per gram of nerve per minute 0.0022 in winter individuals and 0.0034 in summer individuals, an increase of 55 per cent on the part of the summer individual.

3. The rate of carbon dioxide production by the lateral-line nerve of the dogfish averages 0.0024 mgm. of gas per gram of nerve per minute. This rate is slightly less than that of frog nerve.

4. In rates of carbon dioxide production the distal portion and the proximal portion of the lateral-line nerve of the dogfish are indistinguishable. There is no evidence of a metabolic gradient in this nerve.

5. The *nerve fibers* of the lateral-line nerve of the dogfish discharge carbon dioxide at a rate of from 0.0033 to 0.0040 mgm. per gram of nerve fibers per minute or at a rate some 66 per cent higher than that of the nerve as a whole.

6. One centimeter of nerve fiber from the lateral-line nerve of the dogfish has a volume of about 0.001 cu. mm. At rest this discharges 1.1×10^{-8} mgm. carbon dioxide per minute. On stimulation the carbon dioxide output is increased by 1.7×10^{-9} mgm. Each impulse in a series of nervous impulses passing over one centimeter of such a nerve fiber liberates approximately 2.8×10^{-13} mgm. of carbon dioxide, or about 3,850,000 molecules of that gas.

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THE INORGANIC PHOSPHORUS OF FROG MUSCLE IN RELATION TO LACTACIDOGEN AND PHOSPHOCREATINE

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The concentration of inorganic phosphorus in muscle has received a great deal of attention since Embden (Embden, Griesbach and Schmitz, 1914) proposed lactacidogen as a precursor of phosphorus and lactic acid. As a consequence, any change in the amount of inorganic phosphorus appeared to represent an alteration in the amounts of the precursor, to which was assigned the structure of a hexose phosphate.

The importance of phosphorus in muscle has been recently newly emphasized by the work of Eggleton and Eggleton (1927a) and Fiske and Subarrow (1927, 1928). They found that the true inorganic phosphorus is much less than had been formerly given for resting muscle, and that the amount rapidly increases by decomposition of a labile organic compound. According to Fiske and Subarrow, the compound is one of phosphoric acid and creatine, designated phosphocreatine.

Eggleton and Eggleton (1927a) had suggested that the particularly labile compound was a hexose phosphate, and although the designation of phosphocreatine is better supported, it is important to consider what would be the relation of the new facts to the older evidence for the characteristics of Embden's lactacidogen. Evidently the inorganic phosphorus previously determined was really the sum of phosphocreatine and inorganic phosphorus. Irving and Wells (1928) believed that this old method of inorganic phosphorus determination still could be considered appropriate in relation to the lactacidogen theory, and that the lactacidogen and phosphocreatine were quite distinct. But even if they are distinct, phosphocreatine and lactacidogen are related in being organic phosphorus compounds. Where the inorganic phosphorus concentration is small, as in muscle, changes in the principal organic compounds either by release or removal of inorganic phosphorus will have an influence on each other.

The amount of lactacidogen is also affected in muscle hash by lactic acid concentration. Addition of lactate to muscle hash causes a disappearance of inorganic phosphorus which has been considered an indication of synthesis of lactacidogen (Deuticke, 1924). It was suggested by Fiske

and Subarrow (1928) that the decomposition of phosphocreatine into weaker acid constituents would serve as an efficient buffer and neutralizer for the lactic acid produced during contraction. And both Fiske and Subarrow, and Eggleton and Eggleton, have discussed the facts indicating that lactic acid increases and phosphocreatine decomposes at the same time.

It appears to be possible to examine these inferred relations between phosphocreatine and lactacidogen and subject them to some experimental tests. By decomposition of phosphocreatine the inorganic phosphorus concentration is increased, and this change may be brought about by contraction or anaerobiosis. Lactacidogen is considered to participate in an anaerobic stage of metabolism, but its amount is affected by lactate and phosphate concentration. By changing these controllable factors we have compared some of the characteristics of the two important substances (or processes) in muscle, lactacidogen and phosphocreatine, and conclude that they are quite distinct.

Change during muscular contraction. The idea has apparently prevailed that lactacidogen as an intermediary substance would be decreased in amount during muscular contraction and that its expenditure would be indicated in an increased inorganic phosphorus concentration. In view of our present information of phosphocreatine the increase would now be measured as an increase in the sum of phosphocreatine and inorganic phosphorus. With regard to the phosphorus changes in frog muscle during contraction, the principal evidence is from Embden's laboratory (Embden and Hentschel, 1925; Embden and Jost, 1927). Even after short periods of contraction from either single or tetanic stimulation, they found gradually increasing inorganic phosphorus concentration until the total in the contracting muscle was from fourteen to twenty per cent greater than in the resting controls. On the contrary, Parnas and Wagner (1914) and Laquer (1914) had previously been unable to find an increase. In connection with some experiments demonstrating the changes in labile phosphorus (Fiske and Subarrow's phosphocreatine) Eggleton and Eggleton (1927b) remarked that a decrease in the sum of inorganic and labile phosphorus appeared after short periods of work. In reporting experiments on mammalian muscle, Irving (1928) discusses the literature and finds also a frequent decrease in what is now recognised as phosphocreatine plus inorganic phosphorus after short periods of stimulation, results confirmed by Irving and Bastedo (1928).

Because of the conflicting evidence and the fact that the other experiment had not all recognized phosphocreatine, the work was repeated with a series of gastrocnemius muscles from small grass frogs. The results are reported in table 1.

Lactacidogen and phosphocreatine changes in contracting frog muscle. Stimulus was administered by the secondary of an induction coil at varied

TABLE 1

The effect of stimulation on phosphorus compounds of isolated frog muscle

NUMBER	MODE OF STIMULATION	TIME		LOAD	TEMPERATURE	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE	INORGANIC P	P AFTER HYDROLYSIS
		minutes		*grams*	*°C.*	*mgm. per gram*	*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
6	Indirect 30 minutes followed by 3 minute tetanic periods	45	Control	0	4	0.84	0.59	0.35	1.16
			Work	5	4	0.89	0.24	0.65	1.20
5	Direct 60 minutes	48	Control	0	4	0.84	0.59	0.35	1.24
			Work	5	4	0.88	0.19	0.69	1.13
7	Indirect tetanic	10	Control	0	4	0.77	0.52	0.25	1.18
			Work	10	4	0.77	0.40	0.27	1.15
2	10 minutes at 6 per minute, indirect 28 minutes at 30 per minute	38	Control	0	4	0.83	0.72	0.11	1.24
			Work	5	4	0.74	0.14	0.60	1.38
8	55 per minute indirect	10	Control	0	16	0.79	0.57	0.20	
			Work	20	16	0.70	0.20	0.50	
4	Indirect tetanic (interrupted the last 4 minutes)	28	Control	0	4	0.49	0.34	0.15	0.67
			Work	5	4	0.40	0.15	0.25	0.68
10	55 per minute indirect (large muscle)	17	Control	0	16	0.89	0.61	0.28	
			Work	20	16	0.75	0.12	0.63	
9	55 per minute indirect (large muscle)	10	Control	0	16	0.80	0.65	0.15	
			Work	20	16	0.58	0.12	0.46	
3	30 per minute indirect	45	Control	0	4	0.86	0.48	0.38	1.25
			Work	5	4	0.49	0.09	0.40	1.21
11	60 per minute indirect	15	Control	0	16	1.17*	0.66	0.51	
			Work	20	16	1.02*	0.20	0.82	
1	30 per minute indirect	4	Control	0	4	0.71	0.55	0.16	
			Work	5	4	0.76	0.57	0.19	

* The difference in these values as given by gravimetric method was 0.18 mgm. per gram.

The date of experiments: February and March, except nos. 9, 10, 11 during end of April.

rates. Double induction shocks were used, and as a rule the muscle was stimulated through the nerve. Work was done in a moist chamber, anaerobic with nitrogen, and the worked muscle was ballasted with different weights. The control muscle was suspended in a similar moist chamber for the same length of time, having no load. After stimulation the muscles were immediately dropped into liquid air and ground up in mortars. Phosphorus determinations were made colorimetrically by the method of Fiske and Subarrow (1925).

The acid-soluble phosphorus fraction was estimated after one hour extraction in ice-cold 10 per cent trichloracetic acid. The phosphocreatine fraction was calculated by plotting the rate of development of color in the colorimeter cups, against time, and extrapolating to zero time, as suggested by Fiske and Subarrow (1927). The color developed in the unknown and standard simultaneously, so that the ratio of the colorimeter scales showed the actual amount of true inorganic phosphorus in the unknown.

A second portion of the frozen muscle was incubated at 40 degrees for two hours in 2 per cent sodium bicarbonate, and phosphorus determinations were made after extraction in trichloracetic acid. This value represents, according to Embden, the maximum hydrolysis of organic acid-soluble phosphorus, and the difference between it and the maximum color developed in the phosphocreatine determinations is the amount of phosphorus bound in "lactacidogen." The determination after hydrolysis is to be taken cautiously, as the hydrolysis maximum is a rather arbitrary standard, and seems variable to some extent. However, as the value after hydrolysis agrees fairly well in a pair of muscles, an increase in inorganic plus phosphocreatine phosphorus implies a decrease in lactacidogen.

Although the colorimetric method seemed reliable, a set of determinations was made in which colorimetric values and results from Embden's gravimetric method were made on the same sample, and a similar variation was shown by both. This experiment rules out the possibility that labile phosphorus is changed into some form which is not recognizable colorimetrically and yet is included in phosphorus as determined by the gravimetric method.

The series corroborates previously mentioned observations regarding phosphocreatine, in that the value for resting muscle is usually greater than the inorganic phosphorus, and that a decrease in phosphocreatine accompanies anaerobic muscle work. As a result there is an increased inorganic phosphorus value for worked muscles. The inorganic plus phosphocreatine phosphorus, however, shows a decrease after work, except in three cases, two being muscles which were stimulated for a long period, and one in which a very short stimulation was administered.

It is difficult to compare our results directly with those from Embden's

laboratory, for the following reasons: 1. Frogs vary with the season, and most of our experiments were performed with winter frogs. 2. Measurements of the amount of work performed is not attempted: in our experiments the muscles were not exhausted. 3. While our colorimetric determinations are less accurate than Embden's gravimetric, still the changes exceed the chance of error. 4. The muscles in our experiments were immediately fixed in liquid air, while Embden's were usually minced on a cold glass plate before fixation in 4 per cent hydrochloric acid. Considering these differences in method, it is likely that we have a different set of conditions, but the point is clear that under these conditions, with the disappearance of phosphocreatine goes also a utilization of some inorganic phosphorus.

The variation in inorganic plus phosphocreatine phosphorus in these experiments is directly contrary to the observations of Embden, who has obtained under similar circumstances a *gradual* increase in their sum. Eggleton and Eggleton, however, found that some of the inorganic phosphorus liberated from phosphocreatine during contraction disappears, leaving, therefore, a decrease in the sum.

The conception of a "lactacidogen" increase after short periods of contraction is in accord with the law of mass action. The lactacidogen-inorganic phosphorus balance would be driven toward synthesis in short contraction periods on account of the excess free inorganic phosphorus liberated from phosphocreatine and the presence of glycogen and extra lactate for synthesis. When the glycogen supply is diminished by fatigue, it is probable that hydrolysis would predominate.

Lactacidogen and phosphocreatine changes produced by the influence of lactate. The most interesting of the experiments showing the influence of added substances are the ones which show the effect of lactate, because of its occurrence and variation in normal muscle tissue.

Solutions were mixed as required from three stock solutions, isotonic sodium lactate, isotonic sodium bicarbonate, Ringer's solution. In this way different concentrations of lactate can be obtained in isotonic mixtures of sodium lactate and Ringer's solution, and the pH can be controlled as required by the addition of sodium bicarbonate. As the oxygen supply of the muscle would be another variable factor it was likewise controlled.

The first series of experiments (table 2) has to do with the effect of lactate on the inorganic phosphorus content of cut muscle tissue. Three pairs of adductor muscles were cut on an ice cold plate, and immersed in Ringer's solution and Ringer-lactate solutions respectively. The solutions were left exposed to the air at 20°C. for 3 or 4 hours (table 2). The phosphocreatine was completely gone, while in each case the inorganic phosphorus content of the muscle was less in Ringer-lactate than in Ringer's

TABLE 2

The effect of lactate ion on inorganic phosphorus in cut muscles

NUMBER	MUSCLE	TIME	OXYGEN SUPPLY	LACTATE	pH	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE
		hours		*per cent*		*mgm. per gram*	*mgm. per gram*
13	Adductor (cut)	3	Exposed to air only		7.6	0.97	None
				0.3	7.2	0.87	None
12	Adductor (cut)	4	Exposed to air		7.6	1.09	None
				0.3	7.2	0.83	None
14	Adductors—gracilis (cut)	4	Exposed to air		7.6	0.93	None
				0.3	7.2	0.83	None

Date of experiments: March 15.

TABLE 3

The effect of lactate ion on whole gastrocnemius muscles

NUMBER	TIME	OXYGEN SUPPLY	LACTATE	pH	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE	INORGANIC P
	hours minutes		*per cent*		*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
15	3	Aerobic		7.6	1.17	0.32	0.85
			0.3	7.2	1.03	0.18	0.85
16	3	Aerobic		7.6	1.01	0.54	0.47
			0.3	7.2	0.92	0.62	0.30
17	3	Aerobic		7.6	0.96	0.65	0.31
			0.3	7.2	0.79	0.55	0.24
18	5	Aerobic		7.6	0.95	0.65	0.30
			0.3	7.2	0.75	0.47	0.28
19	5	Aerobic		7.6	0.83	0.39	0.44
			0.3	7.6	0.75	0.31	0.44
20	1 40	Anaerobic		6.8	0.94		
			0.5	6.8	0.80		
21	3	Anaerobic		6.8	1.03	0.30	0.73
			0.5	6.8	0.96	0.14	0.82

Date of experiments: nos. 15 to 19, March 17 to 22; nos. 20, 21, April 26.

solution. This confirms Deuticke and Embden in their proposition that lactate ion has a synthesizing influence on phosphate. Muscles used in the experiment were not minced, but merely cut in two or three places; consequently they were allowed more time in the solution than Deuticke used with muscle hash.

It is evident that disappearance of inorganic phosphorus may occur in the absence of phosphocreatine, and it is unlikely that it had any influence before its disappearance. Simple mincing of muscle as practised by Embden, decomposes phosphocreatine immediately, whereas the inorganic phosphorus disappearance occurs slowly.

In the series shown in table 3, whole muscles were used with the object of obtaining a synthesis of lactacidogen under the influence of lactate within the time that phosphocreatine would remain in the muscle. Air was bubbled through both solutions to preserve this latter compound from hydrolysis. A combined phosphorus determination was made for a muscle and the fluid in which it was immersed, so that the total phosphorus of inorganic nature produced by the muscle was measured. Temperature in each case was kept constant at sixteen to eighteen degrees. At the close of the experiments, the muscles were cooled to three degrees and cut in several places to ensure complete extraction. In the last three experiments the muscles were removed from the solution, frozen, and returned to the original fluid to which trichloracetic acid had been added. Complete extraction was obtained by cracking each frozen muscle into several pieces.

In each case there was a greater amount of inorganic plus phosphocreatine phosphorus in the control muscle, than in the one immersed in Ringer-lactate solution, while with one exception the phosphocreatine was hydrolysed to a greater extent in the presence of lactate.

With this one exception, the correspondence between true inorganic phosphorus (last column) in a muscle and its control is remarkable, and would seem to indicate that lactates exert a hydrolysing influence on phosphocreatine to the same extent that lactacidogen is built up, although there is no evidence to show that it is a direct effect. This action is not from pH changes. In the first four experiments there was a small difference in pH, as indicated; but later it was identical in both solutions.

The experiments were repeated with aerobic sartorius muscles which, owing to the fact that there is no heavy, fibrous sheath enclosing them, and also because the muscle is thin, might be expected to permit better diffusion of lactate and oxygen into the muscle tissue (see table 4). The time of exposure, pH, and concentration of lactate were all varied, but the influence of the lactate was not significant in any case. Although there was a tendency toward synthesis in three cases, and no changes were seen in which the opposite effect was shown, the difference in each pair was not

TABLE 4

Effect of lactate ion as shown by sartorius muscles (aerobic)

NUMBER	TIME		OXYGEN SUPPLY	LACTATE	pH	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE	INORGANIC P
	hours	*minutes*		*per cent*		*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
22	2		Aerobic	0.3	7.6	1.13	0.36	0.77
					7.6	1.15	0.33	0.82
23	2		Aerobic	0.3	7.6	1.02	0.26	0.78
					7.6	1.02	0.26	0.76
24	1	15	Aerobic	0.24	7.6	1.05	0.43	0.62
					7.6	0.94	0.42	0.52
25	1	15	Aerobic	0.24	7.6	1.05	0.53	0.52
					7.6	1.06	0.53	0.53
26	1	15	Aerobic	0.5	7.6	0.99	0.39	0.60
					7.6	1.02	0.32	0.70
27	1	45	Aerobic	0.5	7.6	0.92	0.31	0.61
					7.6	0.91	0.31	0.60
28	1	45	Aerobic	0.5	7.6	0.84	0.22	0.62
					7.6	0.89	0.19	0.55
29	2		Aerobic	1.0	7.6	0.95	0.33	0.62
					7.6	0.85	0.30	0.55
30	2		Aerobic	1.0	7.6	0.90	0.32	0.58
					7.6	0.90	0.30	0.60
31	3		Aerobic	1.0	6.8	0.93		
					6.8	0.93		
32	3		Aerobic	1.0	6.8	0.99	0.36	0.62
					6.8	0.87	0.27	0.60
33	2	30	Aerobic	1.0	6.8	0.87	0.24	0.63
					6.8	0.79	0.21	0.58
34	2	30	Aerobic	1.0	6.8	1.02	0.27	0.75
					6.8	1.06	0.23	0.83

Date of experiments: April 2 to 19.

enough to admit a change of phosphorus in the direction of synthesis. The phosphocreatine was similarly constant in corresponding muscles of each pair.

Here we see, then, that with an adequate oxygen supply there is no synthesis of lactacidogen, nor hydrolysis of phosphocreatine, in sartorius muscles under the influence of lactate. Also from these results it is proven that the change in phosphocreatine content seen in table 3 cannot be accounted for by the argument that the muscle is injured by the lactate solution. This would otherwise be a reasonable view, since on immersion in Ringer-lactate solution some muscles show signs of irritation. How-

TABLE 5

Effect of lactate ion as shown by sartorius muscles (anaerobic)

EXPERIMENT NUMBER	TIME	OXYGEN SUPPLY	LACTATE	pH	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE P	INORGANIC P
	hours minutes				*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
35	3	Anaerobic		6.8	1.08	0.21	0.87
			1.0	6.8	0.93	0.10	0.83
36	2	Anaerobic		6.8	0.91	0.25	0.66
			1.0	6.8	0.92	0.16	0.76
37	2	Anaerobic		6.8	0.88	0.35	0.53
			1.0	6.8	0.78	0.23	0.55
38	1 40	Anaerobic		6.8	0.75		
			1.0	6.8	0.58		
39	50	Anaerobic		6.8	1.11	0.34	0.77
			0.5	6.8	0.92	0.20	0.72

Date of experiments: April 20 to 24.

ever, in well aerated lactate solutions phosphocreatine is maintained as well as in control muscles immersed in Ringer's solution.

Under *anaerobic* conditions, however, sartorius muscles show a definite decomposition of phosphocreatine and synthesis of inorganic phosphorus, so that the latter value remains about constant while phosphocreatine is decomposing (table 5). In fact, anaerobic sartorius muscles show the same tendency as aerated gastrocnemii. The range in time, pH and lactate concentration are the same as for aerobic sartorius experiments. The discrepancy shown in no. 36 may be due to the fact that this pair was suspended in solutions in tubes simply filled with nitrogen and closed off from the atmosphere. These same conditions apply to no. 35, however,

where satisfactory anaerobiosis seems to have been established. In the remainder of the experiments nitrogen was bubbled through the solutions.

The difference between anaerobic and aerobic sartorii was tested again, by immersion of both muscles in lactate solutions, one oxygenated, and the other anaerobic—table 6. Here we have further evidence that lactate ion is more effective for synthesis of lactacidogen under anaerobic con-

TABLE 6

Effect of oxygen supply on sartorius muscles in Ringer-lactate solution

EXPERIMENT NUMBER	MUSCLE	TIME	OXYGEN SUPPLY	LACTATE	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE P	INORGANIC P
		hour		*per cent*	*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
40	Sartorius	1¼	O_2	1.0	1.21	0.40	0.81
			N_2	1.0	1.10	0.23	0.87
41	Sartorius	1	O_2	1.0	1.19	0.43	0.76
			N_2	1.0	1.09	0.21	0.88
42	Sartorius	1	O_2	1.0	0.98	0.38	0.60
			N_2	1.0	0.93	0.11	0.80

Date of experiments: April 30 to May 2.

TABLE 7

Effect of oxygen supply on sartorius muscles in Ringer's solution

EXPERIMENT NUMBER	MUSCLE	TIME	OXYGEN SUPPLY	LACTATE	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE P	INORGANIC P
		hour		*per cent*	*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
43	Sartorius	1	O_2	0.0	1.54	0.64	0.90
			N_2	0.0	1.62	0.44	1.18
44	Sartorius	1	O_2	0.0	1.29	0.55	0.74
			N_2	0.0	1.32	0.40	0.92

Date of experiments: May 3.

ditions, possibly on account of the increased amount of inorganic phosphorus from phosphocreatine decompcsition.

In table 7, the effect of anaerobic suspension of sartorius muscles in Ringer's solution is shown, with the control muscle in an oxygenated solution of the same mixture. Phosphocreatine is hydrolysed in the absence of oxygen but there is no suggestion of lactacidogen synthesis in this experiment.

The effect of lactate ion in causing a phosphorus change may be due to either true synthesis of lactacidogen in Ringer-lactate solution, or to arrested decomposition. In many of the experiments it is probable that true synthesis occurs, while in the others the same tendency is shown only so far as to prevent disintegration of "lactacidogen." This conclusion is justified by experiments shown in table 8. In these experiments the control muscles were frozen and extracted immediately in the same solution as that in which the other was immersed and extracted later. With one gastrocnemius a slight synthesis is indicated, while the other experiment shows a small breakdown—but the difference is on the border line of experimental error. The belly muscles show more conclusive results. The Ringer-

TABLE 8

Effect of lactate and Ringer solutions as compared with immediate extraction

EXPERIMENT NUMBER	MUSCLE	TIME	OXYGEN SUPPLY	LACTATE	pH	INORGANIC AND PHOSPHOCREATINE P	PHOSPHOCREATINE P	INORGANIC P
		hours		*per cent*		*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
45	Gastrocnemius	$3\frac{1}{2}$	Aerobic	0.3	7.2	0.70 0.75	0.35 0.15	0.35 0.60
46	Gastrocnemius	$3\frac{1}{2}$	Aerobic	0.3	7.2	0.86 0.83	0.58 0.34	0.26 0.49
47	Belly	2	Aerobic	1.0	6.8	0.92 0.71	0.20 0.12	0.72 0.59
48	Belly	2	Aerobic	None	6.8	0.81 0.86	0.16 0.10	0.65 0.76

Date of experiments: nos. 45 to 46, March 24; nos. 47 to 48, April 23.

lactate solution produced a definite synthesis, while the muscle in Ringer's solution showed a small disintegration of "lactacidogen."

The results seen thus far indicate that the presence of lactate causes a tendency toward the synthesis of lactacidogen,—1. In aerated and anaerobic gastrocnemius muscles. 2. In anaerobic sartorius muscles. Along with this tendency to synthesis, the destruction of phosphocreatine goes in a comparable quantity. That aerated sartorii are in no way affected by lactate, however, leads to the conclusion that when the supply of oxygen is adequate, there is no hydrolysis of phosphocreatine and no synthesis of lactacidogen. This result is not obtained in an aerated gastrocnemius, but these muscles, having a thick fibrous sheath, and

being rounded, are probably anaerobic in many of the fibres at least, in spite of being aerated.

Fletcher and Hopkins (1907) have shown that in muscles which have rested anaerobically, lactic acid is present in increased amounts and that the increase is reduced to normal by exposure to the air. They have also proven that pure oxygen is more effective than air for this purpose.

To show the condition of muscles which have been immersed in lactate solution, as regards their content of lactic acid, eight pairs of gastrocnemius muscles were prepared and one of each pair was placed in an *oxygenated*

TABLE 9

Lactic acid, phosphocreatine and inorganic phosphorus values for muscles immersed in solutions containing lactate

EXPERIMENT NUMBER	MUSCLE	TIME	OXYGEN SUPPLY	LACTATE OF THE IMMERSION FLUID	LACTIC ACID OF MUSCLES	INORGANIC AND PHOSPHO-CREATINE P	PHOSPHO-CREATINE P	INORGANIC P	
								Muscle	Immersion fluid
		hours		*per cent*	*mgm. per gram*	*mgm. per gram*	*mgm. per gram*	*mgm. per gram*	*mgm. per gram*
45	Gastrocnemius	2	O_2	0.5	0.73	0.96	0.78	0.10	0.06
			N_2	0.5	1.22	0.98	0.53	0.32	1.13
48	Gastrocnemius	4	O_2	0.5	2.6	0.74	0.48	0.16	0.10
			N_2	0.5	3.6	0.79	0.25	0.33	0.21
50*	Gastrocnemius	4	O_2	0.0	0.26	1.01	0.69	0.21	0.11
			O_2	0.5	2.7	0.99	0.60	0.26	0.11
47*	Gastrocnemius	3	N_2	0.0	0.73	1.37	0.68	0.53	0.16
			N_2	0.5	1.12	1.20	0.44	0.59	0.17

* Since the solutions in these experiments are not identical, dry substance changes were observed, and the values given here are corrected for this variation.

Date of experiments: May 7 to 22.

solution containing lactate, while the others were immersed in a similar solution bubbled with nitrogen. In the same way two other sets of eight pairs of muscles were compared as shown in table 9, one to show a comparison of lactic acid in muscles resting in Ringer's and Ringer-lactate respectively, both oxygenated, while another shows the effect of these solutions under anaerobic conditions. The muscles were removed from the solution and carefully washed with Ringer's solution to remove any lactate, and found to respond to electrical stimulation. They were then frozen and ground up, and the acid-soluble phosphorus and lactic acid estimated. The phosphoric acid content of the immersion fluid was also determined separately in each case.

Evidently the lactate concentration within the muscles of experiments 45 and 48 is sufficient to provoke synthesis in both aerobic and anaerobic muscles, for the inorganic plus phosphocreatine values are much less than usual. In the last two, synthesis has occurred in both anaerobic muscles. Phosphocreatine concentration is in each case less in the muscles with greater lactate concentration, although the pH of the fluid was the same. Unless, as is quite possible, anaerobic muscles with greater lactate concentration were actually more acid inside, the evidence does not support Fiske and Subbarrow's (1928) suggestion that phosphocreatine decomposes to neutralize lactic acid.

It appears that the amount of phosphorus diffusing from the muscle agrees with the two factors of oxygen supply and inorganic phosphorus concentration in muscle. In fact, phosphocreatine and lactate are evidently related through the oxygen supply. As we suppose that lactate requires oxygen for its removal, lactate may be considered as contributing to the anaerobiosis of muscle by its oxygen consumption. Knowing the close dependence of phosphocreatine upon oxygen supply for its maintenance, we can reasonably suggest that lactates contribute to its decomposition through their reduction of oxygen tension. While such a relation does not alter the facts given by Fiske indicating the buffering power of decomposing phosphocreatine, it is apparent that the complicating conditions of oxygen supply may be equally influential.

Discussion. 1. Lactate ion shows a pronounced disintegrating action on phosphocreatine, *if the muscle is anaerobic.* It has been shown that in anaerobiosis, a resting muscle loses its phosphocreatine, and it may well be that the whole action of lactate on phosphocreatine is a matter of oxygen supply. When lactate diffuses into a muscle it is oxidised to some extent, and thereby utilizes the available oxygen, creating a lesser oxygen tension than would be found in a muscle resting anaerobically in Ringer's solution. This conclusion is supported by the fact that in a thin muscle with an adequate oxygen supply, no hydrolysis of phosphocreatine was detected even in the presence of lactate.

2. The synthetic influence of lactate ion on phosphorus as obtained by Deuticke in muscle hash experiments, is demonstrable in whole muscles, but this action is inhibited by a good supply of oxygen. This may be due to the oxidation of lactate within the muscle, or to a lack of inorganic phosphorus, since phosphocreatine is maintained.

3. The constancy in amount of true inorganic phosphorus when synthesis of lactacidogen and hydrolysis of phosphocreatine is taking place, would lead to the superficial conclusion that these processes are bound together in some way, but if so, the linkage is indirect. "Lactacidogen" synthesis is obtained in muscle hash, with a maximum at a time when the phosphocreatine has completely disappeared. In the same way simple

anaerobiosis breaks down phosphocreatine without any synthesis of lactacidogen. These observations suggest that hydrolysis of phosphocreatine, and synthesis of lactacidogen, are independent processes, but are indirectly related, in that both compounds are in equilibrium with inorganic phosphorus, and that the amount of inorganic phosphorus liberated by the one process may be used in synthesis of the other.

SUMMARY

In the contraction of isolated frog muscles, phosphocreatine is broken down to a value which amounts to as little as one-fifth of the normal content of fresh muscle. Acid-soluble inorganic phosphorus is liberated during this breakdown.

Although in a severely fatigued muscle there may be an increase in the sum of inorganic phosphorus and phosphocreatine, yet during the period in which the muscle is *approaching* fatigue, the increase in free inorganic phosphorus is less than the amount liberated from phosphocreatine; so that a decrease in acid-soluble phosphorus is plainly evident in many cases. This decrease is due to the union of inorganic phosphorus with some organic form to produce an acid-stabile compound, presumably "lactacidogen."

When a muscle is immersed in a solution containing lactate ion, a tendency is shown towards synthesis of "lactacidogen," as seen by the acid-soluble phosphorus decrease. This tendency is restrained if the muscle is sufficiently supplied with oxygen, which also diminishes the lactate concentration in the muscle.

Phosphocreatine is hydrolysed in a solution containing lactate, unless there is a plentiful supply of oxygen available for the muscle.

Added lactate is oxidized to some extent by a muscle, and hydrolysis of phosphocreatine, as induced by lactate, may be due to a more completely anaerobic condition within the muscle, than would exist in a muscle resting in Ringer solution, since some of the oxygen in the muscle would be utilized by this oxidation.

Phosphocreatine decomposes rapidly in anaerobic Ringer's solution. This decomposition is not accompanied by synthesis of lactacidogen; therefore, the two processes are independent, as can be concluded after consideration of muscle hash experiments, where lactacidogen is formed from inorganic phosphorus and a carbohydrate, without participation of phosphocreatine in the reaction.

The compounds may be considered to be in indirect relationship, however, since both are in equilibrium with inorganic phosphorus, and the inorganic phosphorus liberated by the hydrolysis of one, may be used to build up the other.

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STUDIES OF THE EMBRYONIC CIRCULATORY SYSTEM

I. The Influence of H-Ion Concentration and CO_2 on the Yolk-Sac Blood Vessels of the Chick Embryo

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In 1920–21, from observations on the duodenal segment of the albino rat, the thesis was developed that shifts in H-ion concentration are important factors in regulating intestinal contraction and relaxation (1). These experiments paralleled those of Bottazzi (2). They have been confirmed in substance by Evans and Underhill (3), King and Church (4), McSwiney and Newton (5) and others. The later workers, the studies of which are reviewed in the paper of McSwiney and Newton, have extended the observations to other smooth muscle containing organs. The results justify the generalization that H-ion concentration is a physico-chemical conditioner of smooth muscle activity.

In view of these facts we began our studies of the developmental physiology of the circulatory system with an investigation of the reaction of embryonic blood vessels to acid and alkali.

A prime desideratum of all biological investigation is that the test object be as little disturbed from the normal, prior to the actual testing as is possible. Preparatory manipulative interference with normal relations and function is to be avoided. The blood vessels of the yolk-sac of the 4-day chick embryo allow this requirement to be satisfied in high degree. These structures can be observed *in situ* and their reactions measured in the intact living condition with normal blood supply coursing through. In short time experiments, such as those reported here, they are protected from rapid temperature changes and drying by the natural layers of albumen above and yolk beneath. Thus the elements of artificiality introduced by excision, perfusion with solutions of questionable requisiteness, anesthetics, and other customary procedures are eliminated.

Although the histology of the yolk-sac blood vessels has yet to be deciphered, the assumption is justified that embryonic or potential smooth muscle tissue is present. Whether further study establishes this or not, the results reported here are significant.

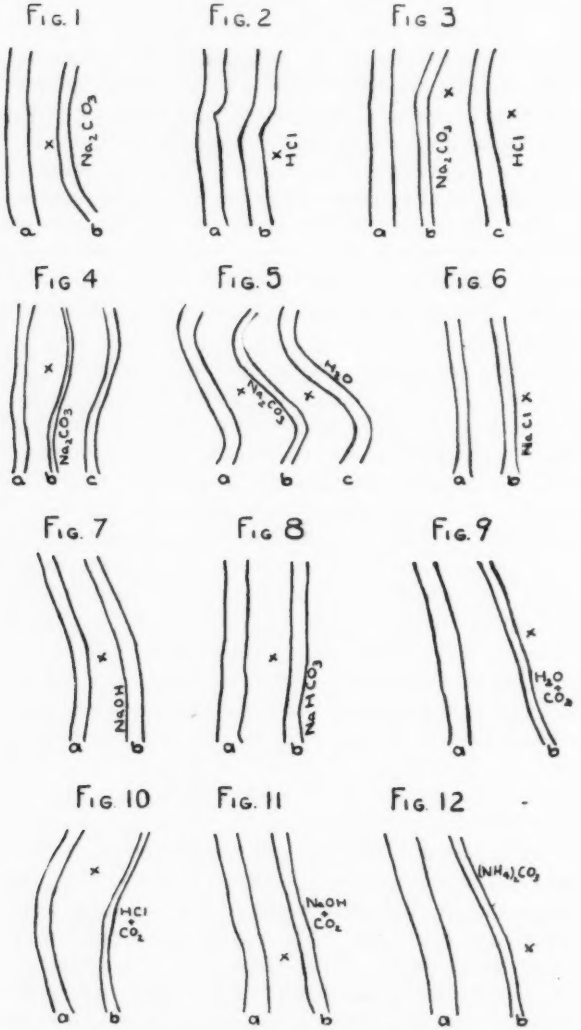
Method. Fresh, clean eggs, not over 24 hours old, from the Pratt

Experimental Farm White Leghorn or Barred Plymouth Rock Breeding Stock, were incubated in a Sears, Roebuck electric incubator for from 96 to 100 hours. This period gave optimum development for our needs. The yolk-sac blood vessel system of the 3-day egg is not sufficiently developed for convenience. In the 5-day egg it is so far along that rupture of the vessels is hard to escape when the shell is being removed. When the eggs were put in the incubator they were marked on the top side, the side to which the embryo gravitates. On removal they were opened on the side opposite the mark so that injury to the sensitive circulatory net work was avoided. Opening was accomplished by cutting out an adequate area (circa $\frac{3}{4}$ inch square) with a safety razor blade or by removing pieces after cracking with a sharp blow. The outer shell membrane was cut through and removed. In a minute or two the yolk-sac rotation brings the embryo to view and at rest.

Support for the egg during manipulation and observation was provided by a wood block ($2\frac{1}{2} \times 4 \times 6$ inches) in which a shallow, egg-shaped depression had been chiseled. This was lined with gauze or cotton when in use. The essential magnification was obtained by the use of a 6× eyepiece and the top lens of a 16 mm. Spencer objective. These were on an old style microscope, the body of which, carrying the coarse and fine adjustments, had been unscrewed from the stage and clamped to an ordinary rod support at a convenient height. The embryo was illuminated and also kept warm from above by a 200 w. Mazda lamp in a suitable reflector attached to the same rod as the microscope. Record of blood vessel size was made by tracing the image reflected from an Abbé camera lucida.

The egg, having been prepared as described, was placed under the microscope and a section of the anterior or posterior vitelline vein was brought into focus and a tracing made. In so far as possible the same section was used in all eggs. Then with a calibrated fine capillary micropipette there was injected into the area contiguous to the drawn section, by puncture just through the yolk-sac, 0.1 cc. of the reagent, the effect of which was to be tested. The injection was so made that the fluid bathed the segment under observation. After a wait of 2 minutes the same section of the same blood vessel was traced to the same scale. One test only was made on each egg. This routine procedure was of course developed after many trials. These showed that the responses to be described are not specific to the vessel finally chosen as representative, but that the smaller channels as well as the larger are reactive. All solutions were made up immediately before testing.

Results. The first tests were made with a Na_2CO_3 solution of pH 9.0. Thirteen tests on 13 eggs gave consistent shrinkage of the vessel (fig. 1, *a* and *b*). This response was pleasing since it tallied with the reaction of smooth muscle to alkalinization.



These figures are tracings from original records. They show a, the size of the blood vessel of the yolk-sac before, and b and c, after the application of the various reagents designated. *x* marks the site of injection.

The next tests (13 in number) were made with HCl of pH 5.0. No valid changes in caliber of the vessels were detectable (fig. 2, *a* and *b*). Here was a result quite contradictory to the expected. For, on the basis of the first tests and the work cited in the introduction, we had anticipated a response by relaxation. The suspicion entered our minds that H-ion concentration might indeed not be a factor conditioning the physiological shifts in size of the embryonic blood vessels of the yolk sac. On the other hand the possibility was present that these vessels were already in a state of extreme relaxation and hence incapable of reaction to added acid.

So 5 tests were made in which Na_2CO_3, pH 9.0 was first used and then after 2 minutes HCl of pH 5.0. Release from the constriction caused by alkalinization followed the acid treatment (fig. 3, *a*, *b*, and *c*). The vessel did not always return to its original diameter, however. While this result was suggestive it was no proof of an acid-relaxing influence. A record was necessary of the state of the alkalinized vessel alone after an interval equal to that occurring when both alkali and acid were successively used.

It was found in 5 tests that recovery from the contraction caused by alkali tended to occur naturally, within 5 minutes, without subsequent addition of HCl (fig. 4, *a*, *b*, and *c*). The same recovery trend obtained when Na_2CO_3 was followed after 2 minutes by distilled H_2O (fig. 5, *a*, *b* and *c*). In neither of these two series did the vessel always return to its original diameter, however.

In order to be sure that this was not due to osmotic phenomena or Na- or Cl-ion, 5 tests were made with 0.85 per cent NaCl. No detectable changes in blood vessel caliber were observed (fig. 6, *a* and *b*).

These results would seem to indicate that the HCl relaxation after Na_2CO_3 constriction is not due to increased H-ion concentration. Corollaries of this are that Na_2CO_3 constriction is not due to OH-ion, and that H-ion concentration is not an important factor in the phenomena observed. The evidence so far, however, is one-sided. It fails to take account of the reaction of the egg constituents and blood of the embryo at this stage of development.

Now both the yolk (6) and the blood (7) of the chick embryo are distinctly on the acid side of neutrality at this time. Moreover the lactic acid content of both albumen and yolk is high in the 4-day chick (8). These facts suggested that the non-relaxing power of the HCl alone and the recovery from the alkali constriction may indeed be due to the inherent acidity of the preparation. If this is so the conclusion that H-ion concentration is a factor is justified. Further experimentation extricated us from the dilemma.

When OH-ions were supplied in excess by NaOH instead of Na_2CO_3 of pH 9.0 no shrinkage of blood vessel caliber occurred in 5 tests (fig. 7,

a and *b*). The same negative response obtained when N/200 NaOH was used in 3 tests, and NH_4OH of pH 9.0 in 6 tests.

This is pretty definite proof that the constriction caused by Na_2CO_3 is not due to alkalinity. From this it can be concluded that H-ion concentration is not the important factor.

The Na-ion and osmotic relations have already been eliminated from consideration. There remains to be tested the hypothesis that the effective agent is the carbonate ion or CO_2.

Six tests with M/10 $NaHCO_3$ gave constriction (fig. 8, *a* and *b*). Seven tests with distilled water saturated with CO_2 gave constriction (fig. 9, *a* and *b*). Six tests with HCl (pH 5.0) saturated with CO_2 gave constriction (fig. 10, *a* and *b*). Five tests with NaOH (pH 9.0) saturated with CO_2 gave constriction (fig. 11, *a* and *b*). Six tests with M/10 $(NH_4)_2CO_3$ gave constriction (fig. 12, *a* and *b*). And even distilled H_2O itself, containing enough CO_2 to make the pH around 6.0 gave weak shrinkage.

TABLE 1

The relation between yolk-sac blood vessel contraction and concentration of Na_2CO_3, $(NH_4)_2CO_3$ and $NaHCO_3$

CONCENTRATION	Na_2CO_3		$(NH_4)_2CO_3$		$NaHCO_3$	
	Number of tests	Per cent contractions	Number of tests	Per cent contractions	Number of tests	Per cent contractions
M/10	10	100	6	100	6	100
M/25	10	100	10	100	10	100
M/50	20	75	20	80	10	40
M/75	10	20	10	20	10	0
M/100	10	0	10	0	10	0

These results are indubitable proof that CO_2 is an agent causing constriction of yolk-sac blood vessels of the 4-day chick embryo. They also are further proof that H-ion concentration is relatively unimportant, since acid (HCl) or alkaline (NaOH) solutions cause constriction only when saturated with CO_2.

DISCUSSION. These observations extend the applicability of Jacobs' (9) hypothesis that the physiological effects of CO_2 are not due to H-ions. His contention that the effects are due to H_2CO_3 or its anhydride CO_2 and not to HCO_3' is also sustained by the fact that CO_2-saturated HCl causes constriction as readily as CO_2-saturated distilled H_2O. It is obvious that but little if any HCO_3' is present in the former solution.

Actual evidence in this direction is afforded when the effective concentration of carbonate and bicarbonate is determined by direct titration on the blood vessels. In table 1 is given the percentage number of responses to $(NH_4)_2CO_3$, Na_2CO_3 and $NaHCO_3$ in decreasing molal concen-

trations. It is seen that although total CO_2 concentration is the same in each of the three solutions at any given molal level, carbonate is effective in higher dilutions than bicarbonate. In the light of modern knowledge of the relative dissociation constants of the two, it is obvious that these results eliminate the HCO_3' ion as the significant constricting agent.

Now although Polansky (10) found no favoring influence of CO_2 on smooth muscle contraction, and Bottazzi (2) records a relaxing effect, our results and the careful studies of Van Dyke and Hastings (11) are in substantial agreement as to the physiological influence of this compound. In view of the highly artificial mode of procedure used in previous studies, save those presented here and those reported by Jacobs, it is obvious that the effects claimed to be primarily due to shifts in H-ion concentration may indeed be due to varying CO_2 concentrations brought about by the changes in acidity and alkalinity of the media bathing the tissue being studied. That is to say, the agent acting directly to produce the phenomena is CO_2, or the carbonate ion, while H-ion is merely one of several factors of indirect influence.

This interpretation seems more logical, particularly when contraction phenomena are in question, since these are determined by protoplasmic consistency and since "there seems to be no doubt that carbon dioxide is an efficient agent for changing the consistency of protoplasm reversibly in either direction . . . ." (12). I am inclined to believe that it is also applicable to respiratory stimulation.

The function of this property of the yolk-sac blood vessels to specifically react to CO_2 by constriction is not at once evident. The property certainly is not accidental. It is based on an intrinsic attribute presumably common to all cells, i.e., the capacity to react reversibly to CO_2 by changes in protoplasmic consistency. Whether or not the property is functionally essential in this particular tissue is at present unanswerable. Any interpretation along this line is of necessity more suggestive than conclusive. It is possible that the property is contributory to the regulation of blood and hence food supply to the developing embryo. It is obvious that temperature variations during incubation under the hen induce variations in mass production of metabolites from which the embryo builds its tissues. With rising temperature this would be accelerated as would the CO_2 production. The latter by its constrictive action on the yolk-sac vessels might well cut down the blood supply to the embryo and thus prevent its being flooded with more food than it could profitably handle. With falling temperature the processes would be reversed, and the relaxed vessels, carrying a larger volume of blood of lesser metabolite concentration would thus provide the embryo with adequate material for uninterrupted mass development. In other words it is conceivable that the maintenance of a proper food supply to the embryo is determined

in part by the CO_2 sensitivity of the yolk-sac blood vessels. This concept is of course purely speculative; nevertheless it has its elements of credibility.[1]

It is probable that the property under discussion is of significance at the time when the yolk-sac is drawn into the chick. This happens on the 19th day of incubation. Lillie (13) is of the opinion that it is "by contraction of the inner walls of the allantois and amnion, that the yolk is pressed into the body cavity." But the yolk-sac at this time "is still a voluminous organ, and equal to about one-sixth the weight of the embryo" (14). Furthermore the yolk-sac membrane is largely a vascular organ the vessels of which are gorged with rapidly flowing blood. This can be seen in the living condition under appropriate magnification. Something more than simple compression would seem to be necessary to reduce this mass of blood vessels to a functionless, limp network preparatory to its being drawn into the chick. Now according to Aggazzotti (15) the CO_2 percentage of the air-sac starts its upward climb on the 12th day of incubation and sharply reaches its maximum on the 18th. Combining this fact with our observations it would seem obvious that the marked CO_2 increase participates in the putting out of commission of the yolk-sac blood vessels prior to the withdrawal of the yolk into the embryo, through the specific response of the vessels thereto by constriction, thus facilitating the response to compression exerted by the allantois and amnion. It may further be that the contraction of the smooth muscle of these organs exhibited at this time is also initiated and brought to its purpose by the increased CO_2 concentration.

CONCLUSION

Using the yolk-sac blood vessels of the living 4-day chick embryo it was found that the caliber of these is not detectably influenced by acid (pH 5.0) or alkali (pH 9.0).

The vessels are, however, specifically reactive to CO_2. The response is one of constriction.

The bearings of these findings on current theories and the developmental physiology of the chick are discussed.

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STUDIES ON SUPRARENAL INSUFFICIENCY

I. The Effect of Suprarenal Insufficiency on Reproduction and the Oestrous Cycle in the Albino Rat

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A variety of observations has, for a very long time, lent support to the theory that the suprarenal glands, especially the cortical tissue, are related to the growth, development and functions of the sexual organs. One group of observations indicates that tumors or hypertrophies of the suprarenal cortex are associated with disturbances in the development of the gonads and of secondary sexual characteristics. Another group of observations, made on a variety of animals including representatives of nearly all the classes of vertebrates, not excepting man, associates hypertrophy of the suprarenals with various events of the reproductive cycle, such as oestrus, ovulation, pregnancy and lactation. For instance, Riddle (1923a) demonstrated a marked increase in the weight of the suprarenal glands of pigeons coincident with each ovulation period and expressed the opinion that both medulla and cortex share in the hypertrophy. A review and discussion of the literature on the subject may be found in Riddle's paper and on page 260 of *Internal Secretion and the Ductless Glands* by Vincent (1925).

Donaldson (1924) found no increase in weight of the suprarenal glands during pregnancy and lactation in normal, healthy albino rats. Lewis (1923) stated that in doubly suprarenalectomized rats "reproductive functions, fertilization, pregnancy and parturition are apparently normal," and that "pregnancy and parturition have been repeatedly observed." Lewis' observations have been frequently quoted. In view of the fact that suprarenal insufficiency may vary markedly in degree after the removal of both glands from albino rats, due to the variable amount of accessory cortical tissue which these animals possess, and stimulated by some helpful suggestions from Dr. H. L. Jaffe of New York, the following investigation was undertaken.

Ability to reproduce after double suprarenalectomy. Twenty-eight doubly suprarenalectomized female rats were exposed to males. Fourteen

were exposed at about two weeks after operation and fourteen from one to two months after operation. Of the latter group, four had been suprarenalectomized at about one month of age. All of the others were operated when adult. Fifteen rats became pregnant and thirteen gave birth to normal litters of from three to seven young. The other two rats died, presumably of chronic suprarenal insufficiency, one day after giving birth to two dead young and ninety-three days after operation in one case, and on the seventeenth day of pregnancy (five fetuses), seventy-eight days after operation in the other case. No gross accessory suprarenal tissue was found in these rats at autopsy and the pathological findings were characteristic of suprarenal insufficiency. The thirteen that had normal litters were killed from two to four months after suprarenalectomy and at autopsy eleven were found to have gross accessory cortical tissue and one had a mass of tissue which was regarded as a doubtful accessory. Only one was without gross accessory tissue.

Of the thirteen that did not become pregnant, four died of subacute suprarenal insufficiency within two weeks after exposure to the males and two died of chronic insufficiency within three weeks after exposure. The other seven were left with the males from two to three months and were then killed. At autopsy three of these were found to have gross accessory cortical tissue and one had one small doubtful accessory.

These results suggested that ability to reproduce after double suprarenalectomy is correlated in a general way with the degree of suprarenal sufficiency remaining after operation. Only three cases out of fifteen indicated that pregnancy is possible in the absence of gross accessory cortical tissue. In these, microscopic accessory tissue might have been present, at least up to the time of conception.

Oestrus after double suprarenalectomy. In a series of forty-one female rats of breeding age, selected from a mixed stock of colored varieties of the albino rat, daily vaginal smears were studied both before and after double suprarenalectomy by the posterior route. The technique and designation of the stages of the oestrous cycle were the same as those developed by Long and Evans (1922). The animals were kept in a warm, well-ventilated room and received careful attention as to hygienic details and a complete diet (Belding and Wyman, 1926). After operation the rats were kept in individual cages and fed on a bread and milk diet. Care was taken to gentle the animals so that they quickly came to show no fear or discomfort during the process of making the smears. In each case the oestrous cycles were studied for two or three weeks before operation and only those rats showing normal cycles were selected for use. The total average length of cycle before operation was 4.9 days, which agrees with the normal length of cycle as established by other investigators.

In order to control for operative trauma, the vaginal smears were

TABLE 1

RAT NUMBER	
300	○○○−− ○○−−− ○○−−− ○○DA′−−− ○ dead
11	○−− ○○○○− ○○○○DA−−− ○○○ dead
190	−− ○○−− ○○○−− ○○○−−−DA′−−−−−− dead
4	○○−−− ○○−−−−DA−−−−−−−−−−−−− dead
1	−− ○○−− ○○−−− ○○○−−−DA′−−−−−−− dead
70	○○−− ○○−− ○○−− ○○○−DA″−−−−−−−−− dead
2	○○−− ○○−−− ○○DA−−−−−−−−−−−−−−−− dead
5	−−− ○○○−−−−−DA−−−−−−−−−−−− dead
40	−− ○○○− ○○○− ○○○−−−−DA′−−−−−−−−−−− dead
10	−−− ○○○−−− ○○−− ○○−DA″−−−−−−−−−−−−−−−−dead
169	−−−−− ○○−− ○○−−− ○○DA−−−−−−−−−−−−−−−−−−− − dead
50	○−−− ○○○−−− ○○○−− ○○DA′−−−−−−−−−−−−−−−−− dead
16	−−−−−− ○○○−− ○○○−− ○○−−DA−−−−−−−−−−−− dead
208	○○−−− ○○−−− ○○−−− ○○DA′−−− ○−− dead
62	○○−−− ○○○○− ○○○− ○○− ○○○DA ○○−− ○○−−−−−−−−− −−−dead
12	○○−− ○○○−− ○○○−− ○○○− ○○DA−− ○○−−−−−−−−−− −−−−−−−− dead
14	−−− ○○○−−−−−DA−−−− ○○−−−−−−−−−−−−−−− dead
19	− ○○−− ○○○−− ○○○−−−DA″− ○○○○○○○ dead
13	− ○○−−−− ○○−−− ○○○−− ○○○DA− ○○−−−−−−−−−−− −− ○○−−−− ○○−−−−−−−−−−−−−−−−− dead
25	○−−−− ○○○−−− ○○○− ○○○− ○DA− ○○−− ○○○−−− ○○○ −−− ○○○−− ○○−− ○○−−−−−−−−−−−−−−−−−−−− −−− ○○−−−−−−−− ○○−−−−−−−− dead
20	○○−−− ○○−− ○○○○− ○○−− ○○DA ○○○−−−−−−− ○○○○ −−−−−−−− ○○−− ○○−−− ○○−−−−−−− ○○○○−−− ○ −−−−−−−−−−−−−−−− ○○−−−− ○○− ○○−−−−−−− −− dead
3	−− ○○−−−− ○○○DA−−− ○○○○−−−−− ○○○−−−−−−− −−−−M−−− ○−−−− ○−−− ○○−− ○○○−−−−−−− ○−− −−−−−−−−−−−−−− dead
24	−−−− ○− ○○−−− ○○−− ○○−− ○DA− ○○○−− ○○○−−−−− −− ○○○○−−− ○○○−− ○○−− ○○−−−− ○○−−−− ○○−−− −− ○○○○−−−−−−−− ○○○−−−−−− ○○−−−−−−− −−−−−− dead
21	○○−−− ○○○−− ○○○− ○○○○− ○DA ○○○○− ○○○−− ○−− ○○ ○−− ○○− ○○○−−−−−− ○○−− ○○−− ○○○− ○○−− ○○−− −− ○○− ○○−− ○○−−− ○−− ○○−− ○○−−−−−−−−−− ○ ○−− ○○ dead
30	○−−− ○○−− ○○−−−− ○○○DA′−−−−−−−−−−−−−−−−− −−−−− ○○○−−−−−M− ○○−−−−−−−−−−−−−−−−− −−−L6 K 89 days CA

TABLE 1—*Concluded*

RAT NUMBER	
309	○○○−−−○○−−−○○−−DA″−−−−−−−−−−−−−−−−−−−−
	−−−−−−−−−−−M−○○○−−−−−−○○−−−−−−−−−−−
	−−−−−−−○○−−−○○−−−−−−−−○○−−−−−○○−− K
	136 days CA
29	○○−−○○○○−○○○−−○○○−○○DA−−○○−−−−○○−−−−−
	−−○−−−−−○○○−−−−−−○○−−−−−−−−○○−○○○−
	−−○○−○○○−−○○○−−○○−−−○○−−−−−−○○○−−−○
	○−○○−−○○○○−−−○○−−○ K
22	−−−○○○−○○○−−○○○○−−○○DA−−○○−−−○○○○−−○○
	−−−○−−−○○−−−○○−○○−−−○○○○−−−−○○−−−○○
	○−−−−−−○○−−−○−−−○○−−○−−−−○−−−−−−○○−
	−−−−−−−−○○○−−−○−−−○ K
23	○○−○−−○○○−−○○○−○○○○○DA−−○○○−−○○○○−−○○
	−−−○○−−○−−−−−−−○○−−−−○−−−−−−−○○−−−○
	○−−−−−−−○○−−−○○−−−−−−○−−−−○−−−○○○−
	−−−−○○−○○−−−−○○−−○ K

In this and in subsequent tables circles (○) represent days of oestrus (stages I, II, III and IV) and dashes (−) represent days of dioestrus (stage V). The letters have the following meanings: DA, double suprarenalectomy; SA, single suprarenalectomy; BO, blank operation; M, exposed to male; L, litter born, with number of young; K, killed for autopsy, with number of days after suprarenalectomy; CA, gross accessory cortical tissue found at autopsy.

studied in a series of twelve rats before and after the removal of one suprarenal gland and exposure and handling of the other in ten cases, and a blank operation consisting of exposure of both glands and the removal of a piece of fat from the kidney region in two cases. Later these rats were doubly suprarenalectomized and included in the total experimental group. In this series the total average length of cycle before the control operation was 4.9 days and after operation it was 4.5 days. Study of individual cases showed that the loss of one suprarenal gland or a blank operation did not disturb the regular occurrence of vaginal oestrous changes (table 2).

Reference to the tables will clarify the following account of the oestrous changes after double suprarenalectomy. The terminology used indicating the degree of insufficiency as shown by the time of survival after operation is that of Jaffe (1926).

Twenty-three or 56.1 per cent of the forty-one doubly suprarenalectomized rats died within one month after operation. Eight of these died of acute suprarenal insufficiency during the first two weeks and fifteen died of subacute insufficiency during the second two weeks. Three of these may be disregarded because of the short period of survival (nos. 300,

11, table 1; no. 101, table 2). Eleven showed no oestrous changes in the vaginal smears from the time of operation until death (nos. 190 to 16, table 1). Five had one oestrous cycle shortly after the operation followed

TABLE 2

RAT NUMBER	
101	– ○○– – ○○– – – ○○○– – ○SA″– – – ○– – ○○– – – – – ○○– – – – – ○○DA– – – – ○ dead
63	○○○– – – ○○○– – ○BO ○– – ○○○– – – ○○– – – DA′– ○○– dead
43	○– – ○○○– – ○○○–SA ○○– – ○○○– – ○○– – ○DA″– ○○– – – ○○○– dead
60	○○○– – – ○○○– – ○SA ○– – – ○○– – – ○○– – ○DA″ ○○– ○○○○ – – ○○○– – – – ○○ dead
18	– ○○○– – – ○○○○○○SA– – ○○○– – ○○○○○○–DA″– – – – ○○ ○○– – – ○○– – – – – – – ○○○– dead
17	○– – – ○– ○○○– – –SA– ○○– – ○○– – – – – ○○DA″– – – – ○○○ – ○– – ○○– – – – ○– – – ○– – – – – – – ○ M dead 77 days
80	– – – ○○○– – ○○○–SA ○○○– – ○○○○○○– – ○DA″ ○– – – ○○○ – – – ○○– – – ○– – – ○○– – ○○– – – – – – ○– – ○○– – – ○ M L2 dead 93 days
6	○○○– – ○○○– – – –SA– ○○○– – ○○○– – ○○–DA″ ○– – – – ○○ – – – – – – – – – – ○○– – – – – ○○– – – ○– – – ○– – ○○○– M L4 K 124 days
7	– – ○○○– – ○○– – ○SA– – ○○○– – ○○– – ○○○DA″– – ○○– – ○ ○○– – ○○○– – – – – – – – – – – – – ○○– – – ○○○– – ○○○–M L4 K 110 days CA
99	○○○– – ○○– – – ○○– – – ○SA″– – – – – – – – – – ○○– – – ○○– – – DA– – – ○○○– – – – – ○○○– – – – – – – – – – ○○– – – – – ○ ○M ○– ○○○– ○○○– – – – – – – – ○○○– – – – – – ○○○– – – K 112 days CA
27	– ○○– – ○○– – ○○–SA– ○○– – ○○○– – ○○– –DA″ ○○○– – ○○ – – ○○○– – ○○○– ○○– – ○○– – ○○– – ○○– – ○○○– ○○M K 124 days
15	○○○– – ○○– – ○○–BO– ○○○○– – – ○○– – ○○DA″– – ○– – ○○ – – ○○– – – ○○– – ○○– – ○– – ○○– – – – ○– – ○○– – – ○M L5 K 117 days CA
33	DA at 35 days. Smear started at 76 days. – – – – – – – – – – – – – – – – – – M K 104 days
64	DA at 28 days. Smear started at 64 days. ○○– – – – ○○○○M L6 K 96 days CA

by complete inhibition of vaginal oestrous changes (nos. 208 to 14, table 1; no. 63, table 2). In four there was no marked change in the occurrence of oestrous changes during the short period that they survived (no. 19, table 1; nos. 43 to 18, table 2). Hence, in sixteen of this group suffering

from acute or subacute suprarenal insufficiency there was practically complete inhibition of vaginal oestrous changes.

Eight or 19.5 per cent of the series died of chronic suprarenal insufficiency within three months after operation, the majority of these deaths occurring between two and one-half and three months. In four of these there were frequent prolonged dioestrous pauses lasting from seven to twenty-four days, interspersed with a few cycles of normal length (nos. 13 to 20, table 1; no. 17, table 2). Rat 17 was exposed to a male but she died twenty days later without becoming pregnant. Two had occasional prolonged dioestrous pauses of from five to fifteen days, with an especially long dioestrous pause preceding death when suprarenal insufficiency was presumably most severe (nos. 3 and 24, table 1). Rat 3 was exposed to a male, became pregnant, but died on the seventeenth day of pregnancy. Two showed practically no significant changes in the occurrence of vaginal oestrous changes after operation (no. 21, table 1; no. 80, table 2). Rat 80 was exposed to a male, became pregnant and delivered two dead young, and died the next day. Hence, in six out of this group of eight rats dying of chronic suprarenal insufficiency there was marked prolongation of the dioestrous pauses. It may be significant that three of the four exhibiting frequent prolonged dioestrous pauses died in from one and a half to two and a half months after operation, while three of the other four died about three months after operation.

Ten or 24.4 per cent of the series of doubly suprarenalectomized rats survived until they were killed for autopsy from three to four and a half months after operation. During this time they showed none of the clinical effects of suprarenal insufficiency. Seven of this group were found to have gross accessory masses, consisting of cortical tissue, at or near the suprarenal sites. These rats may be considered as biologically normal animals, at least during the latter part of their life after the accessory masses of tissue had become established as functional cortical glands. The three which did not have gross accessory suprarenal tissue were probably suffering from chronic suprarenal insufficiency of unknown degree. Two of these surviving rats (nos. 30 and 309, table 1) had prolonged dioestrous pauses, showing no oestrous changes in the vaginal smears for twenty-three and thirty-one days after operation respectively. Later they were exposed to males and no. 30 had a normal litter of six young. Rat 309 did not become pregnant. Both were found to have accessory cortical tissue at autopsy. The early inhibition of oestrus may have been during a period of insufficiency when the accessory tissue was becoming established. The other eight surviving rats (nos. 29 to 23, table 1; nos. 6 to 15, table 2) had no serious disturbance in the occurrence of oestrous cycles. In five of these the cycles were occasionally prolonged to ten or eleven days due to exceptionally long dioestrous pauses. Three had

regular cycles of normal length. The prolongation of dioestrous pauses varied in occurrence in different cases (see tables) and may have been associated with fluctuations in the efficiency of any accessory suprarenal tissue which may have been present. It may be noted that the majority of the prolonged cycles occurred soon after operation when accessory material may have been in the process of becoming established as functional tissue. Gross accessory cortical tissue was found in five cases and no accessory tissue was found in the other three cases. The five that had accessory tissue had been exposed to males and three had become pregnant and delivered normal litters. To summarize: of ten rats which were presumably biologically normal animals, due to the presence of gross accessory cortical tissue or chronically insufficient to a slight degree, eight showed no serious disturbance in the regular occurrence of vaginal oestrous changes.

Two rats which had been doubly suprarenalectomized at thirty-five and twenty-eight days of age were smeared, beginning at seventy-six and sixty-four days of age respectively (nos. 33 and 64, table 2). The vagina had opened on the seventy-fourth and the sixty-third day of age. Rat 33 showed no oestrous changes in the vaginal smear for eighteen days. She was then exposed to a male. After forty-two days with the male she had not become pregnant. She was killed one hundred and eleven days after operation, and no gross accessory suprarenal tissue was found at autopsy. Rat 64 showed oestrous changes in the smear. She was exposed to a male, became pregnant immediately, and delivered a normal litter of six young. She was killed one hundred and three days after operation, and a large, irregular mass of accessory cortical tissue was found at autopsy.

It may be assumed that the absence of vaginal oestrous changes after suprarenalectomy is accompanied by absence of ovulation. The correspondence between the ovary and the genital tract was conclusively demonstrated by Long and Evans (1922). An attempt was made to check this point conclusively by the use of a selective vital dye for the corpora lutea according to the method described by Long and Evans (1922). Four rats were given intraperitoneal injections of 2 cc. of a 1 per cent solution of Dianil Blue 2R on three successive days, and double suprarenalectomy was done two or three days after the last injection. These animals died of acute or subacute suprarenal insufficiency eleven, eighteen, twenty-one and twenty-nine days after operation. In two cases there were no oestrous changes in the vaginal smears after operation and in two there was one cycle followed by complete inhibition of oestrous changes. In all four cases the absence or number present of unstained corpora lutea found in the ovaries at autopsy corresponded with the vaginal oestrous history. Persistent dioestrus after suprarenalectomy as shown by the vaginal smear is due, therefore, to suspension of ovulation.

CONCLUSIONS AND DISCUSSION. The above results indicate that although normal reproduction may be observed in albino rats after double suprarenalectomy, it occurs in animals which have gross accessory cortical tissue and which, therefore, may be considered normal rats from the biological point of view. In a very few cases pregnancy may occur in rats which later die of chronic suprarenal insufficiency but it does not go on to successful parturition. It is most probable that in these cases conception is possible because of the presence of accessory tissue which later becomes physiologically inadequate.

In rats suffering from suprarenal insufficiency there may be partial or complete inhibition of vaginal oestrus changes due to suspension of ovulation. The degree of disturbance of the oestrous cycles is correlated with the severity of suprarenal insufficiency after operation. In the great majority of animals which die of acute or subacute insufficiency there is practically complete inhibition of oestrous. In the majority of those which die of chronic insufficiency there is marked prolongation of the di-oestrous pauses. In those which survive due to the presence of accessory cortical tissue there is no serious disturbance of the oestrous cycles.

These results cannot be taken as conclusive evidence to support the theory that there is a direct relation between the functioning of the suprarenal glands and the gonads. It is well known that in suprarenal insufficiency in rats there is loss of weight, emaciation, asthenia, lowered resistance to toxins and infections, and other symptoms which are evidence of a profound metabolic disturbance. Lee (1925, 1926a) has pointed out that any condition of loss of weight, decreased resistance to infection, undernutrition, extreme variations in temperature, or other serious disturbance of bodily health and metabolism, is quickly reflected in a prolonged dioestrous interval in the oestrous cycle. He demonstrated prolongation of oestrous cycles following thyroidectomy (1925), thyro-parathyroidectomy and parathyroidectomy (1926a). He considered the changes to be due to the effects of removal of these glands on metabolism, rather than as a direct specific effect on ovarian activity. It seems highly probable that the disturbance of ovarian function in suprarenal insufficiency is due to such an indirect effect. Twelve of the rats in the experimental series described in this paper were weighed twice a week during the period from operation to death. Those dying of acute or subacute insufficiency showed a progressive loss of weight up to about 20 grams at the time of death. One which died of chronic insufficiency during the second month after operation lost 5 grams. Those which died of chronic insufficiency during the third and fourth months after operation gained weight very slowly, with many fluctuations, showing a net gain of from 30 to 50 grams at the time of death. Those which survived gained weight steadily from the time of operation to the time when they were killed for

autopsy, showing net gains of from 60 to 101 grams. It may be seen that these results are correlated with the oestrous history as described above, and may be taken as evidence of a metabolic disturbance which might account for the disturbance of ovarian function.

Lee (1926b) demonstrated a lengthening of the oestrous cycle in rats during exposure to low environmental temperatures, and attributed it to the lowered general metabolism, shown by lowered body temperature and lessened activity. It is known that rats suffering from suprarenal insufficiency have difficulty in maintaining their body temperature in cold environments (Belding and Wyman, 1926). Although the rats in the experimental series were kept in a warm room, in which the temperature was regulated by means of electric heaters, it was thought worth while to investigate the body temperature of some of the suprarenalectomized rats. Bi-weekly readings of the rectal temperatures were taken with a certified clinical thermometer on seven of the rats in the series. The total average temperature, calculated from 112 readings on all seven rats, was 97.65°F., a figure which is only slightly below normal. The temperatures in individual cases fluctuated a few degrees, but not to an extent sufficient to account for the disturbances in the oestrous cycles.

Another factor which might account for at least a part of the disturbance of ovarian function in suprarenal insufficiency is lowered blood sugar. Numerous investigators have demonstrated lowered blood sugar in suprarenal insufficiency in a variety of animals. Riddle and Honeywell (1923) showed that each ovulation period in the pigeon is accompanied by an increase in blood sugar, and that the curve describing blood sugar values is essentially similar to the curve for suprarenal hypertrophy at ovulation. Riddle (1923a) suggested that this might be evidence that the relationship between the suprarenal and the ovary is effected through the action of the medullary secretion on the carbohydrate metabolism. He had previously shown (Riddle, 1923b) that ovulation is suppressed in pigeons by injections of insulin. A research on the blood sugar of rats after suprarenalectomy is now in progress in this laboratory.

SUMMARY

1. Of twenty-eight doubly suprarenalectomized rats which were exposed to males, fifteen became pregnant and thirteen had normal litters. At autopsy eleven of these rats were found to have gross accessory cortical tissue. Of the thirteen that did not become pregnant six died of suprarenal insufficiency and three of the seven that survived had gross accessory cortical tissue.

2. Single suprarenalectomy or a blank operation did not disturb the regular occurrence of oestrous cycles.

3. Forty-one female rats of breeding age were studied by the vaginal smear method both before and after double suprarenalectomy. In sixteen out of twenty-three rats which died of acute or subacute suprarenal insufficiency there was practically complete inhibition of oestrus after operation.

4. In six out of eight rats which died of chronic suprarenal insufficiency there was marked prolongation of the dioestrous pauses after operation.

5. In eight out of ten rats which survived until they were killed for autopsy from three to four and a half months after operation there was no serious disturbance in the occurrence of oestrous cycles. Seven of this group of ten were found to have gross accessory cortical tissue.

6. The use of a vital dye selective for the corpora lutea showed that persistent dioestrus after suprarenalectomy as shown by the vaginal smear is due to suspension of ovulation.

7. Weight changes after suprarenalectomy are correlated with the clinical findings and the oestrous history, indicating a disturbance of metabolism.

8. Temperature changes after suprarenalectomy in rats which are kept in a warm room are not sufficient to account for the disturbance in the oestrous cycles.

9. It is concluded that suprarenal insufficiency in rats results in partial or complete inhibition of oestrus, the degree of disturbance of ovarian function being correlated with the severity of the insufficiency.

10. It is suggested that the relationship between the functioning of the suprarenal glands and the gonads is not direct but that it is indirect through the mediation of other factors, probably metabolic in nature.

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THE VALUE OF ACTEROL (IRRADIATED ERGOSTEROL) IN THE TREATMENT OF THYROPARATHYROIDECTOMIZED DOGS

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In some previous work the author reported favorable results in the treatment of parathyroidectomized dogs with cod liver oil (1) and cod liver oil and yeast (2). It was thought that not only the vitamin content of cod liver oil was an important factor, but also the action of the oil in depressing the gastro-intestinal tract might increase the absorption of calcium.

The preparation of irradiated ergosterol[1] containing the active principle of vitamin D made it possible for us to use this product in treating parathyroidectomized dogs. By giving the acterol subcutaneously, or intramuscularly, the action of fats on the intestinal tract is eliminated and the results are obviously dependent on the vitamin action of the irradiated product.

Ten dogs have been used in this study. The diet consisted daily of from 400 to 500 cc. of milk, 130 to 150 grams of kibbled dog cake, and 250 grams of hamburger twice a week. The blood was drawn from either the external saphenous vein or heart. Serum calcium estimations were made by the method of Kramer and Tisdall, revised by Collip and Clark (3). The acterol which was used only after operation is so standardized that 1 cc. is equal to 100 cc. of cod liver oil. The dose used was 0.4 cc. which corresponded to twice the amount of cod liver oil employed in previous work. If tetany developed the dose was given twice daily. Tetany in these animals receiving the acterol was delayed in onset and ameliorated in severity but the acterol did not prevent the serum calcium from dropping to the tetanic level. After twenty to forty days serum calcium returned to normal in six of the seven animals given acterol from the day of operation and no further symptoms of tetany developed. In the animals that refused their food, milk was given by stomach tube. In three animals acterol was not given until after tetany had developed. In the first one, 1 cc. was given intramuscularly and 200 cc. of milk were given by stomach tube. Tetany was quite severe and in one hour after this procedure the

[1] Acterol was kindly supplied by Mead, Johnson & Co.

symptoms were only slightly ameliorated. Two hours later 1 cc. more of acterol was given intramuscularly without the milk. The condition of the animal appeared better in about an hour, but during the night he died. A second animal in very severe tetany was given 1 cc. of acterol in 10 cc. of normal saline intravenously and 500 cc. of milk by stomach tube. In one hour all clonic and tonic spasms had disappeared and the animal lay quietly. In an hour and a half the respiratory rate had dropped from the tetanic level of 230 per minute to 24 per minute. The animal at this time was able to move about with no disturbance of the gait. In a third dog in tetany, the milk given was not retained and 1 cc. of acterol was given intramuscularly with no benefit. An hour later, 1 cc. more was given and 400 cc. of milk by stomach tube. In two hours the animal appeared better and more active. The temperature during tetany was 106.3 and the respiratory rate was 156 but both returned to normal in four hours. It would seem from these results that acterol cannot in itself relieve tetany, but when given in conjunction with milk, very desirable results are obtained. Although milk alone will not prevent or relieve tetany, it is necessary in the diet to furnish the calcium supply for the tissues. This fact has been observed in all the animals as well as these ten given acterol.

In the seven remaining animals given acterol from the day of operation, tetany was less severe than in those just mentioned. Six of these animals made a recovery in fifteen to thirty days. These had mild tetany on the third to the fifth days which was controlled by giving twice daily 0.4 cc. of acterol subcutaneously and 500 cc. of milk by stomach tube. One of the seven given acterol from the day of operation, had very severe convulsions and died on the fourth day. He had been unable to retain any milk for two days and acterol in dosage of 1 to 2 cc. could not influence the tetany. The remaining six animals after fifteen to thirty days, depending on their condition, were not given acterol and no more tetany appeared except in two which developed distemper and died of bronchial pneumonia. This only leaves four for permanent recovery but the two dying of pneumonia had shown no tetany for several days and were receiving no acterol. There was a gastro-intestinal upset in the dogs having pneumonia so that milk could not be retained and acterol was inefficacious to relieve the muscle spasticity that developed. This is further evidence that a calcium supply is necessary for acterol to be effective (although milk alone in large amounts will not prevent or relieve tetany). This same observation was made when cod liver oil was used in treating parathyroid tetany and the results of the two studies compare favorably. The animals apparently recovered from tetany more quickly when given acterol than when given cod liver oil and one animal needed no further treatment after fifteen days.

It would appear from these results that the fats in the cod liver oil probably play no essential rôle in relieving tetany by their depressant action on the gastro-intestinal tract. The active and potent factor in relieving tetany seems to be vitamin D.

During the course of this investigation an article appeared by Urechia and Popoviciu (4) in which they gave ergosterol (Merck) to 8 parathyroidectomized dogs, either immediately after the operation or after the first symptom of tetany. One of these animals given ergosterol from the day of operation lived three months in subacute tetany. The other was still living after two months. Tetany appeared about the third day in these animals. The other six animals given acterol only after tetany occurred died within two weeks. The serum calcium in the first two rose from 6.2 to 9.5 mgm. and 5.9 to 7.9 mgm. during the month of ergosterol treatment and fell again when the ergosterol was stopped. The authors concluded that since six out of eight dogs died in less than two weeks ergosterol did not have a constant effect in prolonging life or in keeping calcium and phosphorus normal.

The results reported here confirm the finding of Urechia and Popoviciu that ergosterol increases serum calcium in parathyroidectomized dogs when given for thirty to forty days. Both their and this report show that ergosterol is of little value in relieving actute tetany. Their article does not state whether milk was used in the diet. It would seem that a calcium supply is important for acterol to be efficacious. They do not believe that ergosterol is of any constant value in treating parathyroidectomized dogs but it at least raised serum calcium in two animals. Perhaps, if all the animals had been given ergosterol from the day of operation, the report would have been more favorable for the use of ergosterol in treating parathyroid tetany. Their findings that animals given acterol after the onset of tetany did not prolong life or relieve tetany does not detract from the results reported in this paper showing that acterol when employed from the day of operation in proper dosage enabled 6 of the 7 animals so treated to recover and need no further treatment.

SUMMARY

Acterol in 0.4 cc. dosage when given in conjunction with milk from the day of operation prevents the development of violent tetany. Animals which cannot retain their milk and thus have no source of calcium will show tetany in spite of the acterol. Recovery is quicker in parathyroidectomized dogs given acterol than cod liver oil. The time for recovery ranges between 15 and 30 days for acterol and 30 to 40 days for cod liver oil. It would seem that a rich supply of vitamin D is efficacious in a large percentage of instances in enabling the body to utilize calcium in the disturbed calcium metabolism found in parathyroid tetany.

Protocol. A-5. Female. Airedale. Weight 35 pounds. Acterol 0.4 daily.

April 23. Thyroparathyroidectomy
April 24. Good condition. Ate all food.
April 25. 8:00 a.m. Paralysis of all extremities. Animal unable to stand. In about 2 hours she attempted to get up but was only able to move her legs in an uncoördinated manner. Blood calcium 6.9 mgm. No muscle tremors.
12:00 Animal passed large black tarry stool.
April 26. 8:00 a.m. Able to walk stiff legged but falls repeatedly. Respiration 165 per minute. Increased salivation.
2:00 p.m. Blood calcium, 5.2 mgm.
Blood coagulation, 7 minutes
Respiration 156
Temperature 106.3
1 cc. acterol intramuscularly and 400 cc. milk by stomach tube.
April 27. No tetany but anorexia present. 500 cc. milk by stomach tube. 0.4 cc. acterol subcutaneously.
April 28 to 29. Good condition. Ate food.
April 30. Blood coagulation time 3½ minutes. No tetany.
April 30 to May 2. Acterol daily. No tetany.
May 3. Blood coagulation time 5 minutes. Active and playful.
May 3 to 7. Fine condition.
May 7. Blood coagulation time 3 minutes.
Blood calcium, 10.0 mgm.
Complete recovery. Acterol stopped. No tetany developed later.

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NOTE ON THE CHANGE OF BLOOD PRESSURE AFTER SECTION OF THE VAGI

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Although the changes in the heart rate and the arterial blood pressure which occur after section of the vagi have been claimed to be independent phenomena by many workers (1), (2), (3), (4), there are several of the standard texts (5), (6) which ascribe the rise in blood pressure which usually takes place, to an increased output of the heart due to its more rapid rate. Marshall (7) showed that the minute volume in the unanesthetized, unoperated, dog is not altered by large increases in pulse rate and Tappan and Torrey (8) found that changes in the pulse rate due to vagus influence did not affect the heart output in dogs under morphine but in the majority of cases did cause a change in dogs under urethane. Other similar observations have been recorded (9), (10), (11), (12). Rutherford (1) believed that the blood pressure change depended on the condition of the visceral blood vessels at the time of section, no rise occurring in fasting animals when the visceral vessels were already constricted. Pavlov (2) could not confirm these results and thought that a rise occurred in animals having a high arterial pressure at the time of section and a fall in those with low pressure. Reed (3) described several different types of responses depending upon the afferent impulses passing in the vagi, a rise usually taking place in animals in good condition and a fall in those in a state of shock. McDowell (4) found that in animals with a low venous pressure produced by hemorrhage, alcohol, histamine, or experimental obstruction, section of the vagi caused a fall in pressure independent of any increase or decrease in heart rate.

The effects of freezing the vagi before and after giving atropine were compared in two series of dogs. The animals in one group were given only morphine (125 mgm.) and those in the other were given morphine (32 mgm.) and ether. The apparatus employed by Binger, Brow and Branch (13), in studying changes in the respiratory rate was used. By cooling to −5°C., the nerves could be completely blocked with minimum stimulation and apparently returned to normal by warming to 37°C. The mean arterial blood pressure was recorded on a kymograph by a mercury manometer connected to a cannula in the femoral artery. Continuous records

were taken before, during and after freezing. The freezing was maintained until the blood pressure level was constant.

Freezing the vagi six times in four dogs under morphine gave blood pressure rises of 22 to 30 mm. and increases in pulse rate of 88 to 145 beats per minute. After giving 1½ mgm. of atropine intravenously, freezing the vagi twelve times in the same four dogs gave pressure rises of 6 to 30 mm.

TABLE 1

Effect of freezing vagi in dogs under morphine (125 mgm.)

		MEAN ARTERIAL BLOOD PRESSURE			PULSE RATE PER MINUTE		
		Before	During	Change	Before	During	Change
		mm. Hg	*mm.* Hg	*mm.* Hg			
Dog V 13 kilo, Male		126	150	+24	58	146	+88
		120	150	+30	60	164	+104
	½ mgm. atropine	126	136	+10	150	220	+70
	1 mgm. atropine	126	136	+10	222	222	0
	1 mgm. atropine	124	130	+6	228	228	0
Dog VI 12.3 kilo Female		118	140	+22	60	205	+145
		115	145	+30	250	245	−5
	1 mgm. atropine	120	130	+10	238	250	+12
		120	130	+10	205	234	+29
Dog VII 14.0 kilo Male		118	140	+22	44	74*	+30
	1 mgm. atropine	110	140	+30	44	82	+38
	1 mgm. atropine	133	141	+8	234	238	+4
		120	133	+13	236	230	−6
Dog VIII 17.6 kilo Male		120	150	+30	60	205	+145
	1 mgm. atropine	120	140	+20	218	218	0
	½ mgm.	110	135	+25	212	218	+6
		100	125	+25	188	235	+47
	1 mgm.	104	126	+22	208	220	+12
	⅔ mgm.	103	125	+22	205	222	+17

* Vagi only partially blocked.

while the pulse rates remained almost constant. The atropine caused increases in pulse rate of 159 to 188 beats per minute with sometimes a slight temporary change in blood pressure. These results are collected in table 1.

With etherized animals the results were less constant. In ten trials upon six dogs under morphine and ether, the blood pressure rose 5 to 22

mm. and the pulse rate increased 0 to 72 beats per minute. There seemed to be some relation between the two as the larger increases in pulse rate coincided in a few cases with the larger increases in blood pressure although in one case in which the heart rate remained constant the arterial pressure rose 10 mm. In nine trials upon four of these etherized dogs each of which had been given $1\frac{1}{2}$ mgm. of atropine the blood pressure rose 0 to 14 mm. while the pulse rate increased only 0 to 8 beats per minute.

In a series of thirty dogs under morphine and ether the vagi were sectioned. In twenty-seven of them the blood pressure rose 2 to 44 mm. In the other three it fell 4 to 15 mm. These three were obviously in poor condition as the pressure before section was under 110 mm. Hg and the heart rate very rapid. In five of the twenty-seven dogs in which the blood pressure rose the pulse rate remained constant or became slower. These results are similar to a larger series reported by Reed (4).

CONCLUSION

The increase of the blood pressure which follows section (or freezing) of the vagi is independent of the increased pulse rate in dogs under morphine and is probably largely independent of it in dogs under morphine and ether.

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LEUCOCYTE CHANGES AFTER ADRENAL REMOVAL

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Variations in the survival period of epinephrectomized animals led us to enquire into the influence of trauma, infection and like conditions. This in turn led to a study of the leucocytes of the blood. In the course of that study it became clear that adrenal insufficiency alone had an effect on the distribution of the white blood cells. The following experiments were directed toward an elucidation of this effect.

The normal range of the total and differential white blood cell count of the cat shows remarkable constancy providing certain factors are taken into consideration. These factors have been found to be similar to those affecting the leucocytes of other forms. Table 1 indicates the percentages of the various types of leucocytes, and shows the normal range of the total white blood cell count to be 5,000 to 15,000. The neutrophiles and the lymphocytes present the same percentage relation as is found in the normal human, 60 to 70 per cent and 20 to 30 per cent respectively.

It has been shown by Menkin (1928) that excitement produces a relative mononucleosis in the cat. Mora, Amtman and Hoffman (1926) have shown that in dogs excitement is followed by an increase in the total leucocyte count. To avoid excitation, therefore, the animal was always handled by the same person. Animals new to the laboratory, however, or to the sampling procedure, became somewhat excited at first, in spite of the precautions taken.

Temperature changes have an effect slightly different from that of emotion. In normal animals cold increases the leucocyte count, but the differential distribution is not affected. In this respect the effect of cold differs from that produced by excitement. It is really quite striking to note the increase in the total count after an animal has been placed for ten minutes or more in a room at 0°. This may amount to as much as 60 per cent of its normal count as shown by cat number 430, table 2 A.

The locus of the mechanism governing this change has not as yet been definitely determined, but in studying the effect of cold on animals from which most of the sympathetic nervous system had been removed, it seems

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quite clear that that system is involved in the change. In animals previously sympathectomized, exposure to cold failed to evoke the rise in the leucocyte count (table 2 B). The total count in these cats was slightly lowered after exposure to cold, but the differential count did not alter appreciably. Although few in number, these cases are given to emphasize the necessity of taking the samples at some constant temperature so as to

TABLE 1

Normal leucocyte counts of the cat

ANIMAL NUMBER	TOTAL WHITE BLOOD CELL COUNT	NEUTROPHILES	EOSINOPHILES	MONOCYTES	LYMPHOCYTES	DEGENERATED CELLS
201		65	4	3	28	
		61	8	3	28	
		59	4	1	36	
322	9,348	76	5	5	10	4
408		64	7	6	23	
		62	12	1	25	
		60	7	1	32	
		62	7	4	27	
		74	3	2	21	
415		67	9	4	20	
416		67	14	3	16	
417		66	5	5	24	
		66	5	6	23	
418		82	6	2	10	
419		64	10	2	24	
420	6,950	63	5	9	23	
	8,300	62	5	8	25	
421	5,400	69	5	5	21	
422	8,800	65	9	2	24	
423	9,850	66	5	6	23	
424	9,400	66	7	2	25	
430	9,150	69	2	3	26	
	12,850	76	4	1	18	1
431*	15,650	70	0	1	28	1
433*	14,300	66	5	6	22	
Average..	5,000 to 15,000	66.8	6.1	3.6	23.3	0.2

* Animals somewhat excited because new to laboratory.

make them comparable. We used a room that varied less than 2° from an average of 25°, and kept the animals in it for half an hour or more before taking a blood sample.

Observations during the course of the experiments have shown that the total and differential counts of the cat are also influenced by wound infections, "snuffles," trauma, and other similar factors. These are associated with a high total and neutrophile count. The total white count may be

TABLE 2

ANIMAL NUMBER	TOTAL WHITE BLOOD CELL COUNT	NEUTROPHILES	EOSINOPHILES	MONOCYTES	LYMPHOCYTES	DEGENERATED CELLS	OTHER CELLS	REMARKS
				A. Effect of cold on leucocytes of normal cats				
430	9,150	69	2	3	26			Normal count
	15,350	68		4	26	2		10 minutes at 0°
	+6,200							
430	12,850	76	4	1	18	1		Normal
	19,300	79	3	5	13			66 minutes at 0°
	+6,300							
431	15,650	70		1	28	1		Normal
	18,750	67	3	2	28			20 minutes at 0°
	+3,100							
432	14,750	30	7	9	52	2		Normal kitten
	14,500	29	9	10	51	1		Normal kitten
	18,050	40	6	3	51			35 minutes at 0°
	+3,550							
433	14,300	66	5	6	22	1		Normal
	19,300	68	5	6	21		2 normo-blasts	60 minutes at 0°
	+5,000							
433	13,900	59	13	3	25			Normal
	17,400	55	15	4	26			20 minutes at 0°
	+3,500							
				B. Effect of cold on sympathectomized cats				
322	18,250	74	7	8	11			3 hours at 25°
	14,950	82	8	1	9			30 minutes at 0°
	−3,300							
322	12,300	76	5	5	10	4		3 hours at 25°
	9,950	72	7	6	13	2		20 minutes at 0°
	−2,350							
328	13,000	49	11	1	39			3 hours at 25°
	9,600	59	7	0	34			60 minutes at 0°
	−3,400							
408	20,050	74	3	2	21			3 hours at 25°
	18,000	73	3	2	22			10 minutes at 0°
	−2,050							

TABLE 3

Effect of infection on the leucocytes of the cat

ANIMAL NUMBER	TOTAL WHITE BLOOD CELL COUNT	NEUTROPHILES	EOSINOPHILES	MONOCYTES	LYMPHOCYTES	DEGENERATED CELLS	REMARKS
342	69,400	83	1	4	12		Severe skin infection
418		82	6	2	10		Very emaciated animal
422	14,200	61	1	7	31		Slight infection and trauma
423	37,200	85		8	7		Intercurrent infection
424	38,950	94		3	2	1	Post-operative infection
427	15,750	85		8	7		Pneumonia
429	14,850	92		2	6		Post-operative infection
Average...	14,000 to 69,000	83.1	1.2	4.9	10.7	0.1	

TABLE 4

Blood counts after the appearance of symptoms of severe adrenal insufficiency

ANIMAL NUMBER	TOTAL WHITE BLOOD CELL COUNT	NEUTROPHILES	EOSINOPHILES	MONOCYTES	LYMPHOCYTES	DEGENERATED CELLS	UNKNOWN CELLS	DAYS AFTER OPERATION COUNT WAS MADE	ANIMAL SURVIVED
Group I. Adrenals removed in two stages									
421	9,700	59	5	2	30	3	1	13 days	13 days, 2 hours
425	7,900	37	5	9	47			4 days	6 days, 0 hour
426	5,400	19	1	2	78			Pre-death	6 days, 22½ hours
428	7,650	53	4	2	39	2		7 days	7 days, 6 hours
433	13,750	52	10	3	35			Pre-death	4 days, 17 hours
434	10,300	44	6	2	43	4	1	8 days	9 days, 12 hours
437	9,800	47	4	3	45	1		Pre-death	12 days, 3 hours
Group II. Both adrenals tied off at one operation									
414		31	8	11	45	5		6 days	6 days, 7 hours
416		43	1	0	56			Pre-death	10 days, 0 hours
417		33	5	7	55			Pre-death	13 days, 2 hours
Group III. One adrenal out, the other badly damaged									
201	11,750	26	3	2	69			8 days	9 days, 0 hours
431	5,250	47	3	1	49			Pre-death	4 days, 22 hours
Average...	5,000 to 14,000	40.9	4.6	3.7	49.3	1.3	0.2		4 to 13 days

200 per cent greater than the normal, while the neutrophiles average 83.1 per cent as compared to the normal average of 66.8 per cent. The changes accompanying infection are shown in table 3.

Removal of the adrenal glands from cats is followed by changes in the leucocyte counts that are quite distinct from those induced by the above enumerated factors. The most marked are those found when the animal shows signs of adrenal insufficiency of fairly long standing. There is a drop in the percentage of polymorphonuclear neutrophiles (average 40.9 per cent) and a rise in the lymphocytes (average 49.3 per cent). The shift in the differential counts of the animals listed in table 4 is more apparent

TABLE 5

Changes in the leucocytes of cat 428 after adrenal removal

DATE	TOTAL	NEUTROPHILES	EOSINOPHILES	MONOCYTES	LYMPHOCYTES	DEGENERATED CELLS	REMARKS
1928							
March 28	20,350	73	1	5	20	1	5 days after right adrenal out
March 29	19,900	71	3	2	24		6 days after right adrenal out
April 4							Removed left adrenal
April 5	28,950	71	9	3	17		First day after both adrenals out
	30,700	70	5	11	12	2	Two hours later
April 6	24,200	62	9	2	27		Spontaneous activity and ate food
April 7	22,200	65	5	2	28		Fair condition, but not interested in food
April 10	17,250	52	3		45		Very weak, could not stand
April 11	7,650	53	4	2	39	2	5 hours before death. 12:45 p.m.
	Even in this extreme condition there is a response to cold, as is shown in the following count which was made after the animal had been put in a room at 0° for 30 minutes. Note that the differential count did not change greatly.						
April 11	27,800	52	5	1	42		After 30 minutes at 0°. 5:00 p.m.

when contrasted with those for normal cats (table 1). This change is not a rapidly occurring or terminal event, but is concomitant with other well-known signs of adrenal insufficiency. It is an absolute as well as a relative variation, because the total leucocyte count does not differ greatly from the normal. The sequence of events is best shown in the following protocol of cat 428 (table 5). Following an operation, the total count rises, the neutrophiles being at the upper limits of the normal. This is associated with trauma and wound healing. Gradually, however, there is a decrease in the neutrophiles and an increase in the lymphocytes. Clinically, this picture is usually associated with chronic wasting diseases such as tuber-

culosis and malaria. Unless upset by external factors or infection, this train of events occurs in epinephrectomized cats, but not in animals subject to other severe operations such as cardiac denervation. It is an additional indication of the extent of adrenal insufficiency symptoms, and occurs in animals showing transient symptoms after sub-total epinephrectomy as well as in those dying after complete adrenal removal. This change in relative percentages of neutrophiles and lymphocytes apparently takes place in the reserve areas as well as in the blood stream. On being exposed to cold, an animal suffering from adrenal insufficiency will respond with an increase in leucocytes, but the differential count remains approximately the same, as shown in table 5.

This white blood cell picture, while apparent in long surviving animals, is easily obscured by infection and shock, particularly the former. Table 3 indicates how greatly the counts during infection differ from the normal (table 1) and from those accompanying the appearance of symptoms of adrenal insufficiency (table 4). These tables give only the end result in each of the cases, the hundreds of counts transitional between the normal and pathological being omitted for the sake of clearness and convenience. One long-surviving animal, 421, had over 40 samples taken and counts made.

In some of the animals the blood picture we had come to associate with adrenal insufficiency failed to appear. Instead there was a rapid rise in the number of leucocytes, particularly the neutrophiles. This phenomenon generally occurs as a result of an acute infection, but we were in most cases unable to find the focus without a complete post-mortem examination. However, all of these animals showed signs of bacterial invasion of some sort. They seemed very susceptible to the infections, as most of them died shortly after removal of both adrenals. Using the blood picture as a criterion, the epinephrectomized cats fell into two groups: 1, those showing signs of some type of infection and dying within a few days (all animals lived at least 36 hours); 2, those showing no infection and living 4 days or more.

All of the animals in the above experiments underwent a thorough post-mortem examination. We are indebted to Dr. H. E. MacMahon of the Pathological Laboratory, Boston City Hospital, for his kind assistance in making these examinations. The results in every case confirmed the blood picture, and many additional cases, not subjected to blood counts, emphasize the conclusions regarding infection. In the near future we expect to report the pathological findings, together with further observations on experiments now under way.

SUMMARY

The normal total and differential leucocyte counts of the cat are given (table 1), together with an enumeration of factors affecting the normal.

Exposure to cold increases the total count, but does not seem to affect the differential (table 2 A). The sympathetic nervous system appears to be related to this leucocyte response to cold (table 2 B).

Removal of the adrenal glands of the cat is followed, in long-surviving animals (table 4, average 8 days), by a shift in the leucocyte distribution—a decrease in the per cent of polymorphonuclear neutrophiles and an increase in the per cent of lymphocytes (small mononuclears). This may be preceded, as shown in table 5, by a post-operative rise of all types of white blood cells, this rise being associated with trauma and wound healing.

Animals surviving complete adrenal removal less than four days, fail to show the typical shift of neutrophiles and lymphocytes, and present instead the blood picture typical of some type of infection, with very high total and neutrophile counts.

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THE EFFECT OF WITHDRAWAL OF DRINKING WATER ON THE SUSCEPTIBILITY OF RATS TO CERTAIN DRUGS

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It is well known that thyroid feeding decreases the minimal lethal dose of morphine for rats (Hunt and Seidell, 1908). It has also been shown by Swingle (1927) that thyroid feeding leads in a sense to dehydration of animals. Kunde (personal communication) has found that the water content of fat-free tissue of animals rendered hyperthyroid from feeding desiccated thyroid is reduced. The question arose as to what effect simple withdrawing of drinking water would have on the susceptibility to morphine. The present paper is a report of the results of withholding water from rats on their susceptibility to a small representative group of drugs.

METHOD. Rats were run in groups of twenty, ten normal stock rats as controls and ten others from the same stock which had received air dried food and no water for 48 (or rarely 72) hours previous to the injection. Doses of the drugs were used which were calculated just not to kill normal rats in the case of morphine and magnesium sulphate, and to kill half of the normal rats in the case of strychnine. The rats were weighed and the equivalent amount of the drug was injected subcutaneously to the left side of the anterior abdominal wall. The two groups of animals received the same treatment after the injections. The time of dying and the total number of fatalities in each experiment were noted. Experiments using pilocarpine, cocaine and atropine were made, but the data were inconclusive and are not included in this report. In the morphine experiments doses of 0.2 mgm. per gram of rat were used. The dose of magnesium sulphate was 1.5 mgm. per gram and of strychnine 0.5 to 0.7 mgm. per gram.

RESULTS. The rats which received no water for 48 hours were much less resistant to morphine than normal rats. In a typical case in which the fatality of the normal rats was 30 per cent, the fatality of the dehydrated ones was 100 per cent. In one experiment a third group of rats was added which had been dehydrated like the others, but two hours before the injections they were given water ad libitum. In this case the fatality was reduced from 100 to 40 per cent. Table 1 gives the complete protocol of this experiment, which is typical for all of the morphine experiments.

The results with magnesium sulphate were almost identical with those

obtained using morphine. In every case more of the dehydrated than normal rats died.

Dehydrated rats seemed less susceptible to strychnine than normal rats. In two experiments more dehydrated than normal rats died, in two the same number of each died, and in three more of normal rats died. The composite percentages show that 58 per cent of the seventy normal rats died, but only 48 per cent of the dehydrated ones.

TABLE 1

Protocol of a typical experiment testing the susceptibility of normal, dehydrated and partially hydrated rats to morphine

	RAT NUMBER	WEIGHT	TOTAL MORPHINE	MILLIGRAMS PER GRAM WEIGHT	RESULTS
		grams	*mgm.*		
Normal rats	1	132	25	0.19	Lived
	2	194	40	0.21	Lived
	3	194	40	0.21	Lived
	4	204	40	0.20	Lived
	5	160	30	0.19	Lived
Dehydrated rats. Water withheld for 48 hours	1	306	60	0.20	Died—5 hours 15 minutes
	2	299	60	0.20	Died—6 hours 30 minutes
	3	330	65	0.19	Died—25 hours 45 minutes
	4	218	45	0.21	Died—7 hours 55 minutes
	5	225	45	0.20	Died—4 hours 40 minutes
Dehydrated for 46 hours, then watered 2 hours before injection	1	290	55	0.19	Lived
	2	274	55	0.20	Died—29 hours
	3	268	55	0.21	Lived
	4	284	55	0.19	Lived
	5	220	45	0.20	Died—5 hours 30 minutes

TABLE 2

Summary of data showing the differential susceptibility of normal and dehydrated rats to morphine, magnesium sulphate and strychnine

EXPERIMENT	NUMBER OF EXPERIMENTS	NORMAL RATS		DEHYDRATED RATS	
		Total number	Per cent fatality	Total number	Per cent fatality
Morphine	3	25	23	25	100
Magnesium sulphate	2	15	25	15	90
Strychnine		70	58	70	48

Table 2 summarizes the results of all the experiments. It will be seen

that the dehydrated rats were more susceptible to morphine and magnesium sulphate, but if anything less susceptible to strychnine.

DISCUSSION. Rats are very susceptible to the absence of drinking water. In a preliminary test nine rats out of ten died after being deprived of water for four days. This occurred in a room where some 200 stock rats were kept none of which died during the period, thereby serving as controls. Rats with no drinking water for two days lose approximately one-third of their body weight. Such animals present roughened body hair, respond tardily, if at all, to external stimuli, and give general signs of "depression."

Morphine, traditionally, is considered a depressant. If this is true, and if the dehydration causes a depression also, then it seems the explanation is that the amount of morphine necessary to kill an animal already depressed is less. However, rats dying from morphine, invariable die after more or less prolonged seizures of convulsions. This lends additional support to the view that morphine possesses stimulating properties, and complicates the simple explanation given above. It is also recognized that the term "depressed" is unsatisfactory, as it does not designate the particular function that is depressed to the lethal point, and it is not accurate to assume that every function of the body is equally depressed.

Magnesium sulphate kills in depression, seemingly without any stimulating actions. The results using this drug, similar to the morphine results, seem more easily adaptable to the explanation of killing a depressed animal with smaller doses of a depressant drug. How much the "tissue dehydrating" action of the magnesium sulphate, from its tendency to create a hydremic plethora from tissue fluids, further accentuates the condition cannot be evaluated from these data.

The question, of course, arises as to whether the lack of water may not be simply "half killing" the animal so that he is more susceptible to any untoward treatment. Strychnine was selected as a drug with stimulating properties to test out this possibility. From table 2 it will be seen that the dehydrated rats are, if anything, less susceptible than normal rats to this drug. There is the criticism that strychnine may not stimulate, but give only the "signs of stimulation" because of its action of decreasing the reflex excitability, primarily of the spinal cord.

The different results with these two types of drugs seem to rule out the alternative that more rapid absorption in the dehydrated animals can be the important factor in killing because of a greater accumulation of the drug at any one time in the blood. If death depended upon a given concentration in the blood, or upon a quick accumulation, then the results using any drug should be in the same direction.

The results suggest that at the depression stage of dehydration, animals are more susceptible to drugs that kill by depression, and less susceptible to drugs which kill by stimulation.

SUMMARY

Withholding drinking water from rats for 48 hours or more increases their susceptibility to morphine and magnesium sulphate, but decreases it to strychnine.

I wish to express my gratitude for the willing technical assistance of Mr. J. Will McBride, in these experiments.

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A MODIFIED HALDANE GAS-ANALYSER FOR USE WITH SMALL VOLUMES OF GAS

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The apparatus described does not introduce any new principles, but follows those of the Haldane apparatus (Haldane, 1912) though it utilises some of Krogh's modifications (1920). It has been designed to analyse mixtures of oxygen and carbon dioxide with a neutral gas, when the volume available is 2.0 cc. or less and when the percentage of any of the gases varies between 0 and 100. It is probably more convenient for such volumes than the micro-methods described by Krogh (1908), Schmit-Jensen (1919) or Christiansen (1925). To facilitate the analysis of varying volumes the graduated burette has been separated from the bulb, and the control tube has been arranged so that the volume contained may be varied and may be made to match that in the analysing system. In use a side-tube, graduated as a burette, is filled with nitrogen and the gas to be analysed is taken into one or more of a series of bulbs; the exact volume introduced is determined by finding the amount of nitrogen that has to be added to make up the full volume of the bulbs used. After absorption of carbon dioxide and oxygen the amount of additional nitrogen that has to be added to make up the initial volume is determined. The side tube containing nitrogen is also employed to wash the gas under analysis into the absorbing tubes, for otherwise absorption is slow since the gases do not mix readily in the narrow bulbs and tubes.

DESCRIPTION OF THE APPARATUS. The apparatus is represented in the figure; it is built so that the whole system is immersed in water. The analyser consists of two tubes, *A* and *B*, each made of 1 mm. bore tubing. *A* has 4 bulbs each of which contains 0.5 cc., not including the volume of stopcock *5*. The volumes need not be exactly the above amounts but the actual volumes contained when the glass is wet must be accurately known. Tube *B* has a length of 500 to 600 mm. graduated in millimeters, and the volume contained in the graduated region is 0.5 to 0.6 cc.; it must be specially selected for evenness of bore and it also must be carefully standardised. The control tube *C* consists of tubing of about 2.0 mm. bore also graduated in millimeters (with a total volume of about 1.2 cc.). Below the water bath the control tube *F* has stopcock *1* fused onto it, by

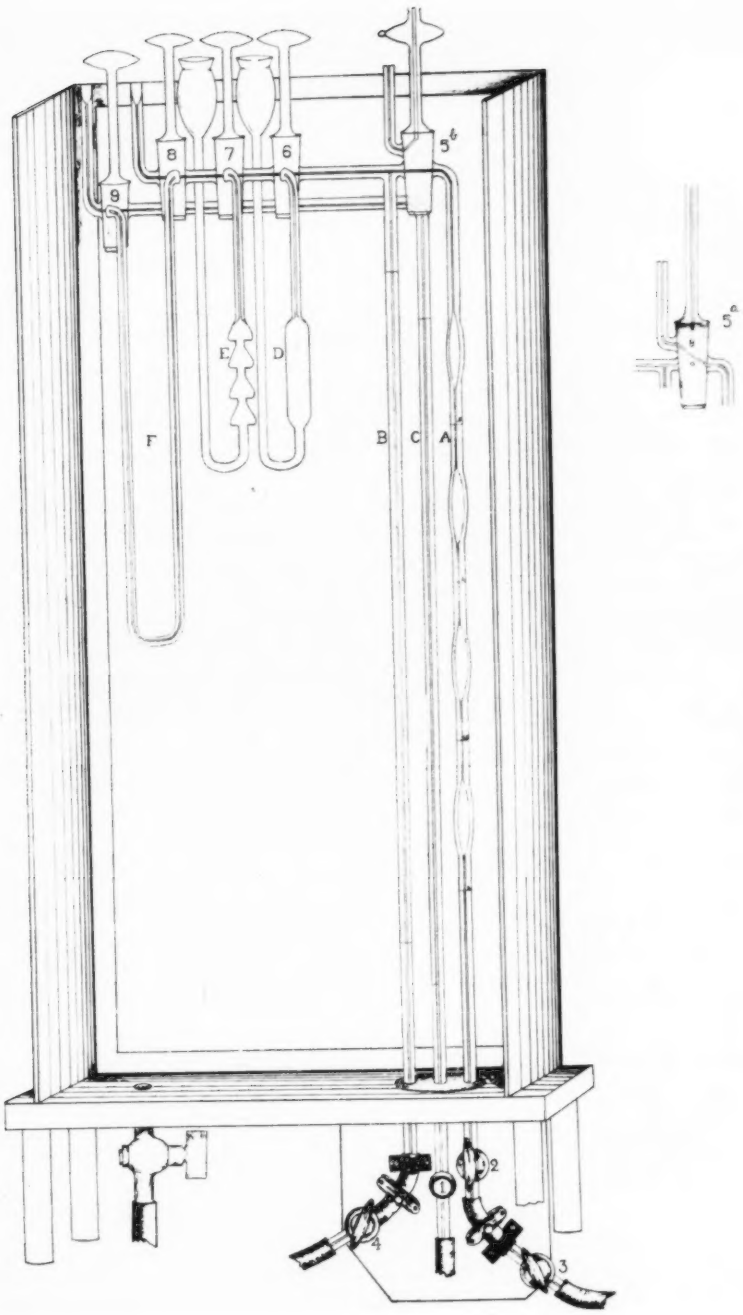

Fig. 1

which the wet mercury which it contains may be set at any definite level with an assurance that this level will not undergo even slight changes except as the result of temperature changes. Tube *A* has stopcock *2* also fused to it; below this stopcock and joined to it by a length of rubber tubing is stopcock *3*. This rubber tubing can be compressed by a screw clip, which after closure of the lower stoptock serves as a fine adjustment of the mercury level in *A*, and the level once set can be fixed by turning stopcock *2*. Stopcock *2* is convenient but not essential. Tube *B* also has stopcock *4* attached to it by rubber tubing provided with a screw clip, which is similarly used for the fine adjustment of its mercury level. Tube *D*, of about 3 cc. capacity, contains 10 per cent caustic potash, and tube *E* contains an oxygen-absorbing solution, protected from contact with air by liquid paraffin. *F* is a U-tube containing kerosene and is used for balancing the analyser against the control tube; it is made of capillary tubing of 0.4 mm. diameter. All the other tubes (with the exception of tubes *A*, *B* and *C*) have a bore of 0.6 mm. Tubes *A*, *B* and *C* are each connected with mercury reservoirs by tubing of pure gum rubber.

The water bath is made of a plate glass front and a back of opal glass, cemented into an aluminium frame-work, and supported on four steel legs. The sides and floor are made of sheet aluminium, and the whole is screwed together and made water-tight with rubber washers. Tubes *A*, *B* and *C* pass through a split rubber cork in the base. The water is stirred by bubbling air through it.

A brass clamp may be screwed on to the heavy base plate of the water bath; it supports a steel rod, which carries the gas sampling tubes of the type pictured in the succeeding paper.

Method of use. The whole system is made absolutely clean and dry. Into control tube *C* some 1 per cent sulphuric acid is introduced so as to moisten it. This is drained out and mercury is used to fill it to any convenient level (see later) and cock *1* is closed. Tubes *A* and *B* are filled with dry mercury, tube *D* with 10 per cent caustic potash and tube *E* with an anthraquinone hydrosulphite oxygen absorber. The actual formula used (which I owe to Dr. E. K. Marshall) is that given in the footnote.[1] Tube *F* is filled with kerosene to any one of a number of graduations.

[1] Formula:

Sodium hydrosulphite (Du Pont's)	16 grams
Potassium hydroxide	14 grams
Sod.-B-anthraquinone sulphonate (Du Pont's)	3 grams
Water	100 cc.

The potassium hydroxide is dissolved and the solution cooled. The hydrosulphite and anthraquinone are added together, the solution shaken for one or two minutes, quickly filtered through cotton and immediately protected from further contact with the air.

Some 1 per cent sulphuric acid is drawn into the top bulb of *A* through cock *5* (position *a*); mercury is run up till it overflows several times, until after standing only a small meniscus of fluid can be seen in *A*. The mercury in *B* is then brought to the top of the tube, and the mercury in *A* with its meniscus of water is slowly raised and is carried through cock *5* (position *b*) until the two columns fuse so that a barely perceptible film of water can be detected on both columns of mercury when these are standing at the tops of their tubes.

Tube *A* is filled with air and, with mercury in *B* standing at the top of the tube, nitrogen is obtained and checked by measuring the remaining gas. It is contained in the bulbs of *A*, and any fraction of a bulb is measured in *B*. The mercury in the control tube *C* should then be set at an appropriate level according to the volume to be analysed. The volume likely to be still present in *A* and *B* at the end of the analysis is roughly estimated, and the nitrogen present in *A* and *B* is reduced to this volume. The setting of control *C* is then determined at which the temperature expansions of air, glass, and, in control tube *C* of the locked mercury column, so balance one another that no apparent expansion or contraction of the nitrogen in tubes *A* and *B* can be detected when the temperature of the water bath is altered one or two degrees. The readings are made after resetting levels in *D* and *E* and rebalancing against *C* through U-tube *F*. In this way a graph may be readily made indicating the optimum setting of the level in *C* for varying volumes of residual nitrogen (it is the last stages of an analysis that proceeds most slowly and in the earlier stages an exact balance is less essential).

Side tube *B* is then filled almost completely with nitrogen and excess nitrogen is expelled though cock *8*, so as to wash out with nitrogen the tube lying between cocks *7* and *8*. Before a sample is introduced, the control tube is opened to the air through cock *9*, and the nitrogen in *A* and *B* is balanced against it; cock *9* is returned to its normal position ⊢, cock *5* is closed in an intermediate position, tube *B* is very carefully balanced against tube *C*, and this level is read giving the initial volume of nitrogen in tube *B*. The nitrogen left at the top of tube *A* is then set at a slightly positive pressure, cock *5* is turned to position *a*, and the mercury reservoir is raised and the remaining nitrogen forced out. The mercury is carried through to the side tube of the sampling tube and air bubbles are carefully disposed of by pinching rubber connections (as described by Haldane, 1912). No washing back and forth of the gas is allowable, either at this time or previously for the purpose of moistening the tubes. The tubes may be kept adequately moist (any excess moisture must be avoided) by moistening the rubber connecting tube between the apparatus and the sampling tube, when the mercury will carry back with it adequate moisture. Small pockets of gas are bound to exist at irreg-

ularities where the tubes connect within the stopcock; if the above procedure is followed and the cock position is exactly set, these remain of approximately standard volume and consist of pure nitrogen. If washing is allowed, their composition is variable and with the small volumes used the error is not negligible.

The volume introduced is kept close to that of one or more bulbs and the pressure is brought as close as possible to atmospheric pressure by adjustment of the sampling tube. Cock *9* is turned to an intermediate position ⊀ to lock the kerosene balance, cock *5* is turned to position *b*, and the level in *A* is then set exactly at one of the graduations, and that in *B* is adjusted till the kerosene level is returned to normal. Cock *9* may then be cautiously reopened, the levels set exactly and the initial volumes read.

Cock *9* is then again closed, cock *6* turned to position ⊢ and carbon dioxide is absorbed. In this the gases in *A* are brought into *D*, and the level of mercury in *A* is raised until it passes through cock *5* and lies just at the edge of tube *B*. The nitrogen in tube *B* is then used to wash on the other gas by bringing up the mercury to the top of the tube. With the mercury in *B* in this position, that in *A* is then used to pump the gases back and forth. When the absorption is complete the readings are made in *A* and *B* as before; *B* is always used to measure any gas which does not fill a bulb completely.

Absorption of oxygen is carried on in a similar manner, and the oxygen left in tube *D* is "fetched" in the usual way. It is difficult to prevent the absorption being very slow in the narrow tubes; absorption can, however, be made less slow if mixing is improved by manipulating tubes *A* and *B* so as to reverse the column of air occasionally. The mercury in *A* is raised till it lies at the top of tube *B*, the last of the gas (containing most oxygen) is then taken into tube *B* and the rest into tube *A*. On return, that in *B* is moved back first, followed by that in *A*, so that oxygen-rich gas is taken as far as possible into *E*.

If the cocks and tubes are free from any partial blocks balancing can be effected very accurately and successive readings should always check within 0.2 mm. of the scale on *B*.

If the volume for analysis is less than 0.5 cc., the volume available is taken into tube *A*, cock *5* is turned to position *b*, taking care that the kerosene is locked by an intermediate position of cock *9*. The mercury in *A* is then lowered to the end of the first bulb while that in *B* is allowed to rise to provide the necessary nitrogen. The level is exactly set in *A* and then that in *B* is adjusted to give approximately atmospheric pressure. Cock *9* is then cautiously opened, an exact balance is struck, readings are made, and the analysis then proceeds as usual.

A simpler model of the analyser may be used; in one such the water

bath was of the kind ordinarily used in a gas analyser and merely contained tubes *A*, *B* and *C* between the cocks. The cocks were all of the simple T type such as those used in the Haldane instrument, and the oxygen and carbon dioxide absorbers were similar to those pictured in the figure but formed mere inverted cups dipping into other cups, as in the Henderson model. With such a machine, fairly accurate results may be obtained if the precautions already mentioned about taking the sample are observed, if the temperature of the room is relatively stable, and if care is taken to diminish the heat given to the machine by the operator. To diminish this temperature error the operator should wear gloves and a fan should be used to blow from behind the apparatus. Even with these precautions results are liable to some error from temperature changes, since a very appreciable portion of the gases is outside the water bath.

Errors and their avoidance. With the partial water bath of the simpler model, temperature changes provide the greatest source of error. Besides the precautions already mentioned the analysis should be completed as rapidly as possible. For this reason the hydrosulphite absorber has advantages, particularly since the low percentage of alkali prevents difficulties arising from dehydration. If a pyrogallate solution is used the gases should always be taken over the 10 per cent caustic potash before the volume is read. The whole analysis may be made in 15 to 20 minutes if the mixing of gases is rapid. This mixing is more satisfactory if the bulbs of A are shorter and rounder than those pictured in the figure, if they lie closer together at the top of tube A, and if the bore of tube A below the lowest bulb is larger. Such changes allow more abrupt movement of the mercury in the bulbs, improving mixing and quickening absorption. Also the more even the bore of the tubes at stopcock junctions, the less likelihood will there be of gas pockets being formed within which oxygen may disappear mainly by diffusion. The rate of gas absorption varies therefore considerably in different samples of the same machine. As a rule oxygen should be removed by some 60 to 120 movements of the mercury column if the absorber is good. Standardisation by dry mercury may be used for tube B; if the mercury is always maintained at the top of the tube, except for brief periods during readings, the main part of the tube is maintained practically dry. On the other hand, the pumping movements spread the fluid throughout the bulbs in A (though most of it remains in the upper bulb) and wet standardisation values should be used. The upper bulb of A can only be standardised empirically by analysis of outside air, for the pockets of gas remaining in crevices in the stopcock do not enter into an analysis and yet will be measured by the ordinary mercury method. The total volume estimated by mercury is useful as a check; it should always be larger than

the effective volume determined as indicated above. All mercury standardisations should be made with fused stopcocks and preferably with the tubes contained within their water bath.

Care must be taken to make certain that the bore of stopcock *5* is sufficiently even that mercury droplets do not stick to it, when mercury is carried through it. If this is not true, it must be cleaned or if necessary be rebored.

A water block may readily form in the finer tubing connecting with the control tube *C;* if the temperature falls considerably, mere condensation water may create such a block, and complete drying of the upper part of the control tube may be necessary to get rid of it.

The mercury in *B* is only in contact with nitrogen and should remain clean for months; that in *A* soon gets dirty and may have to be cleaned after some 10 analyses. It may be cleaned, however, without touching the rest of the apparatus.

It will be noticed in the figure that cock *5* is bored centrally and that this tube connects in position *b* with the tube through which samples are taken in; if by any chance this tube is fouled (by blood or plasma) in introducing the sample of gas into the machine, it may be washed out at once without interfering with the analysis in progress.

ACCURACY OBTAINABLE. An example will illustrate the agreement of readings to be anticipated, and the resulting accuracy.

A gas sample which, when analysed in a 10 cc. Haldane-Henderson apparatus, gave the following results; CO_2 4.96 to 4.97, O_2 15.58 to 15.54, N_2 79.45 to 79.50 per cent, gave in the small analyser the following results:

	VOLUME IN BULB	BURETTE READING	VOLUME IN BURETTE	
Nitrogen.....		0.92	0.5108	Sample 0.4900 − 0.1209 = 0.3691
Sample.....	0.4900	12.39	0.3899	
CO_2 absorbed.....	0.4900	14.12	0.3719	CO_2 0.0179 = 4.85 per cent
CO_2 absorbed.....	0.4900	14.11	0.3720	
O_2 absorbed.....	0.4900	19.54	0.3156	O_2 0.0566 = 15.34 per cent
O_2 absorbed.....	0.4900	19.56	0.3154	

It will be observed that the CO_2 percentage estimated is 0.1 per cent and the O_2 percentage 0.2 per cent too low. The error with a volume of 0.37 cc. should rarely exceed this amount; as a rule the percentage will be underestimated. The dilution of the sample with nearly twice its volume of nitrogen exaggerates errors and also slows gas absorption. The last readings given above only differed by an amount that might be due to error in reading and yet might indicate that absorption was incomplete.

When a larger volume is available a much better result can be obtained. The same gas gave with 2.0 cc. samples the following consecutive values:

VOLUME	CO_2 PERCENTAGE	O_2 PERCENTAGE	N_2 PERCENTAGE
2.0175	4.94	15.62	79.44
2.0140	4.94	15.63	79.43
2.0138	4.95	15.57	79.48
1.9763	4.96	15.61	79.43

The following values were obtained on analysis of outside air with 0.5 cc. samples:

VOLUME	CO_2 PERCENTAGE	O_2 PERCENTAGE	N_2 PERCENTAGE
0.4910	0.02	20.96	79.02
0.4879	0.02	20.91	79.07
0.4910	0.04	20.98	78.98
0.4935	0.04	20.91	79.05

With the simpler machine and only a partial water bath the results are not quite so good even if great care is taken to avoid temperature changes as far as possible. The following consecutive values for outside air were obtained:

VOLUME	CO_2 PERCENTAGE	O_2 PERCENTAGE	N_2 PERCENTAGE
0.5168	0.04	20.88	79.08
0.5186	0.04	20.94	79.02
0.5088	0.10	20.89	79.01
0.5103	0.02	20.92	79.06
0.5053	0.08	20.94	78.98

While the accuracy obtained is fairly good, it must be clearly understood that neither form of the apparatus is to be recommended where large volumes are available for analysis. The small bore tubes introduce considerable difficulties and the prevention of partial blocks is often difficult. It is probable, however, that a 10 or 20 cc. model built on similar lines would prove an accurate and adaptable apparatus.

I should like to take this opportunity of expressing my thanks to my friends and assistants who have made this work possible; to Mr. J. D. Graham, the University glassblower, who not only built the apparatus, but considerably improved its design; to Mr. H. Krieger, our mechanic, who designed and built the water bath; to Mr. A. Afford, our technician, who, though less concerned in the development of this particular apparatus, has reduced the labour of gas analysis through the care he

gives to the machines, and to Dr. L. Sribyatta, for his kindness in drawing the figure.

CONCLUSION

A modified gas analyser is described capable of analysing mixtures of CO_2 and O_2 with a neutral gas in any proportion in volumes up to 2.0 cc. The error varies from ± 0.04 to ± 0.2 per cent according to the volume available for analysis.

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THE EFFECT OF LOCAL CHANGES IN TEMPERATURE ON GAS TENSIONS IN THE TISSUES

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Goldschmidt and Light (1925b) demonstrated that the degree of oxygen saturation of venous blood was not only dependent on the local temperature, but that a minimum saturation was demonstrable at skin temperatures between 20° and 30°, and that above or below these levels high saturations were the rule. They also clarified the physiology of the skin by showing (1925a) that blood, which came mainly from skin vessels could be obtained from the veins on the dorsum of the hand, and that this blood had a much higher oxygen saturation than that from other veins. Bazett and McGlone (1927) demonstrated that the temperatures of the tissues of the limbs were usually low (confirming data of earlier workers) and that the temperatures of the muscles of the forearm at rest rarely exceeded that of the surface by more than 2° to 3°C. Since it had been demonstrated by Stadie and Martin (1924) that blood in vitro, when warmed without change of gas content, had an increased hydrogen ion concentration and higher CO_2 tension, it seemed important to determine acidity changes in vivo. Austin and Cullen (1926) discussed the changes that occur in vitro and pointed out that changes in temperature affected not only pH but also the relation of this to pOH and to the neutral point, and a later study (Austin, Sunderman and Camack 1927) on alligators showed that in the cold-blooded animal none of these were kept constant when the temperature changed, and demonstrated the complexity of the reactions concerned.

Bazett and McGlone (1928) injected phenol red into animals and were able to show that the tissues became alkaline when cooled, and that the rate of acid formation during stasis was much diminished at the lower temperatures. They noted that hyperemic reactions to stasis at varying temperatures were similar in duration and intensity to the degree of acidity. They were only able to demonstrate very slight colour changes in human skin. The technic of air injection into the tissues introduced by Campbell (1923a) appeared well adapted for a quantitative study of such changes in man. Campbell noted that exposure to warmth might raise the CO_2 tension but in a later paper (1923b) he attributed these changes to the effect of the anesthetic. Still later (1925) he noted a fall of CO_2 tension

when the body temperature was raised. His results were obtained in animals injected with large volumes of air; he also obtained (1924) a CO_2 tension under his own skin 1 mm. below that of alveolar air, when 100 cc. of oxygen had been injected.

Fig. 1. The sampling tube arranged as for air removal with a rubber collar protecting the ground joint. The mercury is not represented.

Fig. 3. Minutes after injection are plotted as abscissae and CO_2 and O_2 percentages as ordinates. In this figure (and in figs. 4, 5, 6 and 9) circles denote data obtained on subject B and crosses those obtained on subject S. Curves are drawn through data obtained on S with the forearm in air and with surface temperatures between 33° and 34° and also with the forearm in water at 22°. CO_2 values are indicated by interrupted lines and simple crosses, O_2 values by continuous lines and ringed crosses. The data from subject B were obtained when his surface temperature was also about 33°; here closed circles are used for CO_2 values and open circles for O_2.

Fig. 4. Bath temperatures (or in air experiments skin temperatures) are plotted as abscissae and CO_2 tensions in the tissues in mm. Hg as ordinates. Subjects are distinguished as in figure 3 and a third subject is represented by triangles. Open circles and ringed crosses are questionable.

Fig. 5. The data of figure 4 replotted according to difference in tension between the tissues and alveolar air.

Fig. 6. Bath temperatures are plotted as abscissae and O_2 tensions as ordinates. The upper curve is considered to represent average conditions in B with a high oxygen saturation of venous blood, and the lower curve those in S with a much lower saturation.

Fig. 7. Temperatures of the bath are plotted as abscissae and the differences in temperature between it and the tissue as ordinates. Data obtained with thermocouples at depths of 1 to 4 mm. are indicated with closed circles and at depths of 5 to 6 mm. with open circles. The interrupted line is considered as representing the data at the lesser depth and the continuous line at the greater depth. Data obtained by Harris and Marvin (1927) on the temperature of blood in the veins of the hand immersed in still water are plotted as triangles. All data represent temperatures after at least 30 minutes' immersion, and no distinction is made between different subjects.

Fig. 8. Minutes from immersion of the arm in water are plotted as abscissae, and temperature differences between the tissue and the water as ordinates. All data were obtained in subject S. The uppermost curve represents the temperatures observed at a depth of 4.8 mm. where air had been injected and the limb immersed in water at 10.2°. The interrupted portion of this line represents the rise in temperature during an attempted bleeding. The crosses represent the temperatures observed at a depth of 2.7 mm. when air had been injected and the arm immersed in water at 40.8°. The open circles show the fall of temperature at a depth of 3.4 mm. on immersion of the arm in water at 25° and the lower curve with closed circles the fall at this same depth on changing the temperature of the bath rapidly from 25° to 20°.

Fig. 9. Minutes after removal from the water are plotted as abscissae and actual temperatures observed as ordinates. The depth of the thermocouples was in all cases between 2 and 3 mm. Subjects indicated as in figure 3. The lower two curves indicate the rise of temperature on removal from water at 11.5° and the upper two curves the fall on removal from water at 42.2°. The distinction between the two subjects is only noticeable at the higher temperature.

Fig. 5.

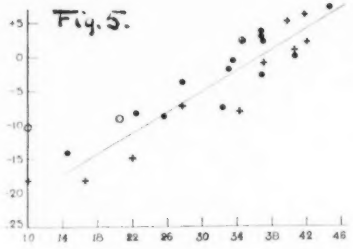
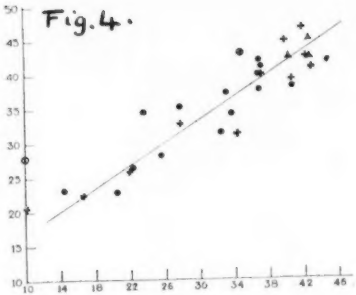


Fig. 4.



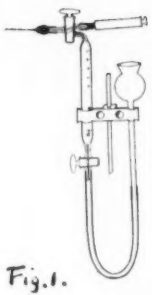


Fig. 1.

Fig. 8.

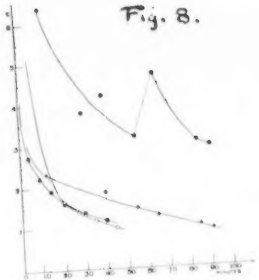
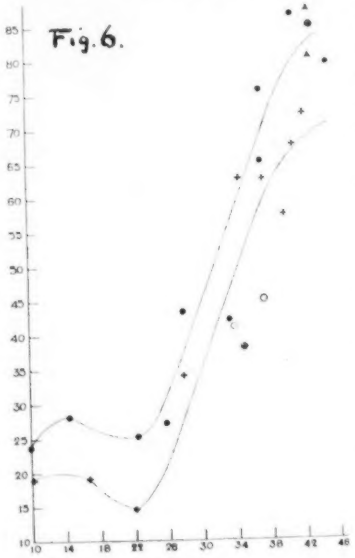


Fig. 6.



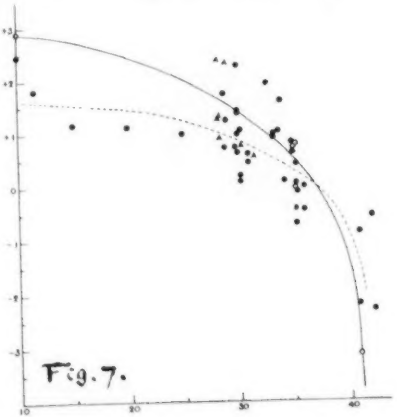


Fig. 7.

Fig. 3.

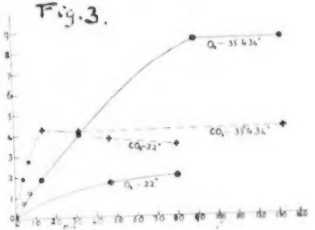
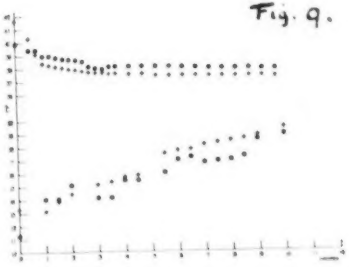


Fig. 9.



METHOD USED. A modified Campbell technic has been employed reducing the gas injected to a volume usually of 20 cc., occasionally of 10 or 40 cc. This has been rendered possible by the development of a modified analyzer capable of giving an adequate analysis of volumes of 0.5 cc. or less. This is described in the preceding paper (Bazett 1928); the analyzer employed was of the simpler type with a partial water jacket. The air injected was usually nitrogen, though in many experiments either alveolar air samples, rebreathed air, or artificial gas mixtures were employed. Samples were removed at periods varying from 70 minutes to over 2 hours and in most experiments two such samples were removed at times differing by a half hour or more, so as to demonstrate definitely that the gases were approximately in equilibrium. The air was injected just beneath the dermis on the back of the forearm through an ordinary hypodermic needle

Fig. 2. X-ray of air injection in subject S, demonstrating varying depth, with some air localized to one superficial area and the rest dispersed in layers at greater depths (1/1).

connected by rubber tubing with special gas sampling tubes filled with mercury. A small glass tube with cotton plugs, sterilized by dry heat, was inserted between the needle and the sampling tube to filter the air. Beyond this glass tube all parts were sterilized in alcohol. The sampling tubes were either of 10 or 20 cc. volume of the shape indicated in figure 1. The connecting tubes were washed out with the gas used and the gas was injected by adjusting the mercury to give an adequate pressure (usually 50 to 60 mm.); the amount injected was noted by the graduation on the tube. The pressure at the end of the injection was measured and was always found to be falling; it was usually between 0 and 20 mm. at the time the needle was withdrawn. The air was removed in a similar manner, except that the dead space was reduced by connecting the needle with the sampling tube directly by a ground joint protected also with a rubber cover. The needle had lateral holes (made by Downing Bros., London); a syringe

attached to the side tube of the sampling bottle was used to withdraw ½ to 1 cc. of gas to wash out the air contained in the needle, since mercury had initially filled the whole system except the needle.

The gas on injection spread out into a thin irregular layer extending from the hand to above the elbow, though most of it remained in the forearm. On the first occasions on which it was injected it often remained fairly localized (see fig. 2) but with repeated injections this became rarer and a thin layer of the type visible in the upper part of the arm in figure 2 is more common. The air particularly tended to follow the sheaths of the superficial veins. The depth was demonstrated by x-rays to be very variable (2 to 10 mm.); the mean depth was probably 4 or 5 mm. If the air remained localised a sample could be withdrawn fairly readily; when it was diffuse, the gases were moved by massage as far as possible to one locality, and maintained there by applying tight rubber bandages above and below. In a few cases 10 cc. of air were injected in the back of the hand; the tension needed for injection was greater, the process was more painful and the gas harder to remove. In this case a rubber glove with a hole on the back made the best method of localizing the air for removal. With injections of 20 cc. into the forearm it was uncommon to be able to withdraw more than 4 cc. of gas after 1 or 2 hours, except in the earlier experiments when the gas remained more localized. The 4 cc. usually consisted of two samples taken at different times and of this 1 to 2 cc. was lost in washing out the needles. On removal the pressure of the gas was apparently rarely above atmospheric pressure; on one occasion it was measured and found to be at this level; at times it has been definitely at a positive pressure, but only after localizing the air by massage and usually after application of rubber bandages.

Some experiments were made to determine the time taken for equilibrium to be attained. Figure 3 shows some data obtained in this way. In these experiments at the higher temperature nitrogen was injected into both arms of subject S and samples were later obtained at intervals. The arms were left in air and the surface temperatures were measured at a number of points on several occasions and averaged. At the lower temperature a single forearm was used. The data on subject B were obtained during the actual injection of nitrogen; during this process the mercury was used to move the nitrogen back and forth (as in absorption in a gas-analyser) and the samples analysed represent the rate at which equilibrium is approached under these conditions. It is evident that equilibrium is attained in about ¼ hour for CO_2 and 1½ hours for O_2, when nitrogen has been injected. If air is injected with an oxygen content above the equilibrium point, the equilibrium is attained much more slowly so that such mixtures were avoided except in the initial experiments. One sample protocol will make this clear.

1:50 p.m. Nitrogen 20 cc. injected into left forearm; pressure on completion of injection 9 mm. Hg. 1:55 p.m. Alveolar air (CO_2 5.0 per cent, O_2 15.35 per cent) injected into right forearm; pressure on completion 6 mm. Hg. 2:06. Both arms put into water at 14.3°C. to level of elbow. 3:14. Air removed from right forearm gave CO_2 3.55 per cent and O_2 11.17 per cent, and 3.50 the same arm gave CO_2 2.45 per cent, O_2 10.87 per cent. 3:20. Left arm gave CO_2 2.99 per cent, O_2 3.79 per cent, and at 4.05 CO_2 3.13 per cent and O_2 3.65 per cent. The temperature of the water at the end was 14.6°C. and an alveolar air sample at this time gave CO_2 5.43 per cent (tension 38.8 mm.) and O_2 14.30 per cent.

It will be noted that in this experiment the oxygen tension on the side nitrogen was injected attained apparent equilibrium in about 90 minutes, while on the other side the O_2 percentage fell slowly during the first $1\frac{1}{4}$ hours and after this fell still more slowly. The progressively slowing fall of oxygen percentage probably can be partly explained as the result of a slowing circulation as the arm became cooled and the blood could probably remove little oxygen, since under these circumstances it must have been already almost fully saturated. When the tissues are warm and the blood flow rapid, the blood is also highly saturated, can give off oxygen more readily than it can absorb it, and even under these conditions equilibrium is more readily obtained from a low than from a high oxygen level.[1]

In the protocol quoted considerable differences may be detected in the CO_2 tensions of the air in the two arms, and on the left side the tension fell considerably during the later part of the experiment. A gradual fall of CO_2 tension could be explained by a progressive fall of temperature in the tissues, as will be seen later. The difference between the two arms as far as CO_2 tension is concerned was 4 to 6 mm. and may have depended on a difference in depth of the air sampled (since even a single injection may exist at varying depths), on varying degrees of inflammatory reaction, or on a definite error in the second sample from the left forearm. This sample (0.365 cc. only) contained some edema fluid. Edema fluid was often seen in the later samples but could generally be sidetracked into the syringe. When this was impossible it created a possible error in the analysis, since any contact with acid might generate extra CO_2. Such errors were later avoided as far as possible by washing the sampling tubes and analyser with distilled water instead of 1 per cent sulphuric, keeping the moisture minimal and regarding possible CO_2 absorption as a lesser error. In the actual experiment quoted the lower CO_2 value would agree best with average results.

The control of temperature has been attained by immersion to the elbow level in water at varying temperatures; the depth was measured and allowance made for the water pressure. The water has been stirred either by a rotating stirrer or by bubbling air continuously through the bath, and the temperature has been controlled either by hand or by a thermostat. In most experiments one arm has been maintained in water at a high and the

[1] If a gas mixture in equilibrium was injected and no change occurred, the tensions so determined agreed well with those obtained by N_2 injection.

other at a low temperature, so as to make the contrast between two experimental temperatures on the same occasion. Under such circumstances the vasodilatation in the warmed or the vasoconstriction in the cooled arm may perhaps be modified slightly as the result of reflex effects from the opposite arm, but the subject is likely to remain more normal and is less subject to violent sweating as the result of the warm bath. The arms have been removed for periods of about 5 minutes during the procedure in order to allow blood or air samples to be taken, and on completion the arm has usually been exposed to the room conditions for 5 to 10 minutes during the withdrawal (usually more difficult) of the final air sample.

Four measurements have been made of the tissue temperatures in actual experiments with air injected; in many other experiments the temperatures existing under the skin under similar conditions have been measured as well as the rate at which these temperatures are attained or lost. For this purpose the loop thermocouples designed by Dr. B. McGlone (described by Bazett and McGlone, 1927) have been employed.

In a few experiments no water bath has been used but the arm has been maintained in air and the surface temperatures have been sampled at intervals as already described. The gas tensions observed under these circumstances showed no consistent variation from the curve obtained with the arms in water.

Comparison has been made with arterial alveolar air samples taken by the Haldane-Priestley (1905) method (using expiratory samples only) obtained usually at the end of the experiment, so as to correspond with the conditions at the time the tissue gases were removed. Two samples were taken, and the CO_2 tensions usually checked within 1 to 2 mm.; the average of these was used. In a few cases only a single sample was taken. Comparison has also been made with blood samples taken from the hand veins, which have been assumed to represent skin blood, and to be more or less comparable with the air injections. However, the hand has rarely been used for air injection and skin blood from the hand cannot be expected always to compare exactly with air from the forearm. The blood samples form an incomplete series since it is not easy to obtain the whole set of data in any single experiment. The data obtained on the blood together with further data obtained in similar experiments without air injection are reported separately in the third paper of this series.

Results obtained. The dependence of the gas tensions on temperature is readily seen in figures 4, 5 and 6. The subjects showed no particular differences in regard to CO_2 tensions, and in both B and S the tensions at these depths were below that of alveolar air at normal skin temperatures. Only when the skin temperature approximated 37° was the alveolar CO_2 tension attained. This difference has been fully established since alveolar air injected under ordinary temperature conditions on withdrawal showed a lower CO_2 percentage.

As regards O_2 tension considerable individual differences were observed, but in both subjects the curves obtained were similar. The data presented in figures 4, 5 and 6 have been calculated after allowing for water vapor tension at the probable tissue temperature, which experiments have demonstrated to be nearly that of the bath. For comparison with blood data more exact information of the tissue temperature is required. Consequently the temperatures existing under these conditions at depths up to 5 mm. beneath the skin have been determined and are plotted in figure 7. The data for temperatures between 29° and 36° were obtained in the course of another series of experiments with immersion of not only the arm but of the whole subject in water; none the less they probably represent a comparable condition. The other data have been obtained partly in actual experiments with air injection and partly in parallel experiments without air injection. The temperature in the tissues differed from that of the water slightly more in experiments where air had been injected and had thickened the subcutaneous tissue, but there were no great differences between the two sets of data. The tissue temperature in any experiment has been assumed to be that estimated by determining the midpoint between the two curves drawn. Since oxygen percentages are only capable of slow modification it is unlikely that warming or cooling of the skin after removal from the bath seriously altered the oxygen percentage of the air removed 5 or 10 minutes later, but it is quite possible that the rate at which oxygen equilibrium is attained depends partly on the rate at which the temperature changes develop. On the other hand CO_2 percentages, which are capable of more rapid changes, may probably also be varied by the rate at which normal temperatures are recovered, while the air is being drawn.

Figure 8 represents the rate at which temperature changes are attained in subject S under varying conditions. A rapid rise in temperature during removal of the arm for bleeding is noticeable. Data obtained in subject B were similar in character except that in hot baths the tissue temperature did not approach as closely to that of the bath as it did in subject S (difference between the two subjects of about 1 to 2°C.) and in warm baths equilibrium appeared to be reached more rapidly (in less than 1 hour).

Figure 9 represents the rate of recovery of more normal temperatures after removal from hot and cold baths. These changes probably caused appreciable variations in CO_2 percentages during the withdrawal of the air samples.

When these various factors are taken into consideration, discrepancies of individual values from the curves in figures 4, 5 and 6 are often readily accounted for. The CO_2 tension of 28 mm. observed in subject B with a water temperature of 10°C. was obtained in a sample which was drawn slowly and with great difficulty and where the tissue temperature must

undoubtedly have risen considerably after removal of the arm from the water. In addition some blood and edema fluid was withdrawn with the sample; even 0.001 cc. of CO_2, if given by this fluid to the air sample on contact with acid, would have exaggerated the CO_2 tension by 2.3 mm. Hg. Both errors would be in the same direction, and the value plotted is probably too high.

The oxygen tension values were fairly regular at extremely low or high temperatures. The aberrant values for subject S of 63 mm. at a temperature of 34.3° and of 38 mm. at a temperature of 34.8° were taken with the arm in air, and the surface temperature over the area injected was estimated from the mean of at least 10 points. The high value of 63 mm. was obtained in an experiment with numerous punctures in an attempted determination of the rate of attainment of equilibrium. At the time the sample was withdrawn the arm was definitely inflamed. The value of 41 mm. at 33.5° for subject B was obtained with the arm in air and surface temperature measurements were used as in the case of S; the low value of 45 mm. recorded for a temperature of 37° for subject B may not represent equilibrium; 20 cc. of nitrogen had been injected 101 minutes earlier but became so diffuse that a second 20 cc. was injected in addition 64 minutes before the sample was taken. Data are not available as to the rate equilibrium is attained under such circumstances. It would appear that there may be real variations in oxygen tension at these intermediate temperatures which cannot all be explained by any observed source of error and which would correspond with the similar variations in oxygen saturation observed by Goldschmidt and Light (1925b). The curves, however, probably represent the conditions existing when the surface temperatures are either below 20° or above 36°, when the circulatory conditions are more constant.

Discussion. *Differences between the two subjects* may depend on previous history as to climate or on racial differences. Subject B is 42 years old and has had the history previously reported (Bazett and McGlone, 1927); subject S is 29 years old, is accustomed to the climate of Siam where the temperature often reaches 48°C. and is humid; he has spent 7 of the last 9 years in America. There is undoubtedly a considerable difference between the subjects; the dermis of subject B appeared in the x-rays and on puncture to be thicker (some 10 to 30 per cent) than in S and on the forearm he is much more thickly covered with hair. The differences in blood composition and in the rapidity of temperature changes are most easily explained by assuming a blood flow through the skin which is more rapid in B than in S at the higher temperatures. The blood from the veins of the back of the hand has always been highly saturated with oxygen in B (over 87 per cent saturation), while in subject S 90 per cent saturation has been the highest observed (water bath 40.8°) and the lowest has been 71 per cent

(water bath 39.6°); saturations of 83 and 85 per cent have observed at bath temperatures as different as 34° and 43°. These samples have been taken a few minutes after withdrawal of the hand from the water as the arrangement of the bath made the holding of the hand in water during bleeding too difficult. There may, therefore, have been some return of vasoconstriction before the blood was taken. Similar contrasts were seen in a few samples taken from arm veins, where subject S under warm room conditions had an oxygen saturation of about 60 per cent or less, while a single sample on B was 87 per cent saturated. The fact that in a bath at 42.2° the subdermal temperature in subject S attained equilibrium at 41.6°, while in B the temperature at a similar depth was 39.9°, is also most easily explained as the result of a faster flow of blood in the skin of B. B was apparently absorbing heat and sweating violently, while S was not particularly uncomfortable. Further evidence of less local protection from heat was the fact that a bath at 43° for $1\frac{3}{4}$ hours produced in S a slight skin inflammation lasting into the next day, while a similar inflammation was only observed in B after immersion in water at 45° for $1\frac{1}{2}$ hours. Tissue temperatures of a little above 42° appear, therefore, to produce a long lasting inflammatory reaction in both subjects. On removal from a hot water bath the tissue temperature fell more rapidly in S than B, but the differences in rate of warming on removal from cold water were not beyond chance variations in conditions (see fig. 9).

The *variations observed in tension of* CO_2 were relatively consistent and did not appear to be affected appreciably by the individual differences in rate of skin circulation already discussed; presumably, therefore, the dominant factor was some physical effect of temperature rather than any variation in velocity of blood flow. The CO_2 tensions observed beneath the skin of the forearm have in many cases been compared with those calculated from blood samples withdrawn on the same occasion from the hand veins. The values calculated have often only differed by a few millimeters of mercury from those observed beneath the skin. The actual data obtained are presented in the third paper of this series. The conditions observed in vitro apparently therefore hold in vivo, and the considerable variations in CO_2 tension indicate a great change in the pH level with temperature (alkalinity at low temperatures) similar to that demonstrated in animals by Bazett and McGlone (1928). The magnitude of these changes is indicated in the following paper. It is possible that the changes in circulation are to some extent dependent on these variations in acidity or CO_2 tension. When the data of figures 7, 8 and 9 are used to estimate the effective tissue temperature for CO_2 tensions with varying bath temperature (allowing for warming of the tissues for five minutes after removal from the water) bath temperatures of 10° correspond with tissue temperatures some 5° or 6° higher and bath temperatures of 42°

with tissue temperatures some 4° lower. The data plotted in figures 4 and 5 may then be corrected to show the relationship to probable tissue temperatures by changing the scale of the abscissae so that 10° to 42° bath temperature represents 15° to 38° tissue temperature.

The effect of the temperature changes on oxygen tension is much more marked since the physical effect of temperature changes, the effect of increased alkalinity on oxygen dissociation and the decreased oxygen saturation at temperatures of 20 to 25° must all aid in lowering oxygen tension as the temperature falls. Below 22° however the oxygen tensions rise, the greater oxygen saturation at the lower temperature demonstrated by Goldschmidt and Light succeeding in overbalancing the physical effects for a short range, below which the physical factors appear to become dominant again. The rise in oxygen tension on lowering the temperatures from 22° to 15° must depend on an increased oxygen saturation of the blood due to deficient oxygen utilization by the tissues. The increased tension excludes a primary retention of oxygen by the hemoglobin. It will be shown in the succeeding paper that when skin blood from hand veins is examined the oxygen tension calculated on theoretical grounds corresponds fairly well with that observed in the tissues at both high and low temperatures.

Changes in oxygen saturation resulting from variations in blood flow and from variations in oxygen usage by the tissues when combined with the physical factors can explain the observed tensions. Rothlin (1920) has shown that high oxygen tensions can cause arterial contractions; in our experiments vasoconstriction is accompanied by low oxygen tensions and there is no evidence that the magnitude of the local circulation is regulated by changes in oxygen tension under these conditions.

The bath temperature at which the oxygen tension is minimal appears to lie (on the small amount of data obtained) between 20° and 25° corresponding well with the data of Goldschmidt and Light (1925b) which gave a minimum saturation at about 25°C. We have no evidence, under the climatic conditions existing in America, of cyanosis at such low bath temperatures (15°) as those indicated in the data presented by Lewis (1927, fig. 41).

The data presented may be of considerable general importance. The temperatures which exist, not only in the fat but also in the muscles, in the limbs at rest are much below 37° as demonstrated by many workers (Bazett and McGlone, 1927). When the temperatures in the muscles are as low as 34° the oxygen tension is likely to be at most 40 mm. (since the blood is likely to be less saturated) and the CO_2 tension in the range of 30 to 35 mm.; during work even if the temperature only rose to 38° the oxygen tension (for a similar saturation) would be raised to 70 or 80 mm. and CO_2 tension to 40 or 45 mm. as the result of the temperature changes alone.

In order, therefore, to obtain a real conception of the interchange of gases in the peripheral muscles temperature changes (both of blood and muscles) must be considered, and should be included if possible in nomograms such as those of L. Henderson and his co-workers. The fact that lactic acid may under some conditions be removed from the blood by resting muscle (Barr and Himwich, 1923) while under other conditions the resting muscle may give up lactic acid to the blood (Himwich, Koskoff and Nakum, 1928) may be partly dependent on the temperature of the muscle.

The fact that the tissue pH and oxygen tension may both be very much affected by local temperature changes, which produce alterations far in excess of the normal differences between arterial and venous blood at the same temperature, may be of great importance in relation to infection. It would certainly appear logical to keep all tissues infected with anaerobic organisms at the highest possible temperature which did not produce tissue damage.

CONCLUSIONS

1. Injection of gas mixtures or of nitrogen beneath the skin of the forearm in 20 cc. volumes, allows an estimate of the tissue tensions of oxygen and carbon dioxide.

2. The method has demonstrated that the effect of baths at different temperatures on the conditions present is considerable and that CO_2 tension is under normal conditions *lower* in the subcutaneous tissue than in alveolar air. Curves are presented showing the relationship of CO_2 tension to the temperature of the surface.

3. Oxygen tension is still more dependent on temperature and in this case a complicated curve is obtained since the variations in oxygen saturation demonstrated by Goldschmidt and Light play an important rôle.

4. The high oxygen saturation of blood at lower temperatures is dependent on a failure of the tissues to utilize oxygen rather than on the physical effects of temperature on hemoglobin dissociation.

5. The relationship of these gas tensions to actual tissue temperatures is discussed and data are presented demonstrating the effect of the baths used on the actual temperature in the subcutaneous tissue.

6. The importance of the results obtained to the fuller understanding of muscle metabolism and their possible value in explaining variations in local resistance to infection are briefly discussed.

We would like to take this opportunity of thanking Doctors Goldschmidt and Austin for valuable advice during this development of their earlier work, as well as to Messrs. W. H. Erb, M. M. Friedman and J. B. Quicksall, second year medical students, who assisted in the preliminary experiments and who would have continued to coöperate in this work if their class schedule had made it possible.

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THE EFFECT OF LOCAL CHANGES IN TEMPERATURE ON BLOOD FROM THE HAND VEINS

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Goldschmidt and Light (1925 a, b) have demonstrated that the blood from the hand veins, mainly derived from the skin, has a different composition from that obtained in the forearm, and they have also shown the effect of baths of different temperature on the oxygen and carbon dixoide content of the blood obtained. The data here presented were obtained by similar methods and are mainly of interest in that the blood was drawn from the same subjects (usually in the same experiments), on which the measurements of tissue gas tensions reported in the preceding paper were made. They therefore allow some estimate to be formed of the dependence of these tensions on the blood composition.

METHODS USED. The hand and forearm were immersed in water as described in the previous paper. Usually one arm was held in cold and the other in warm water. After 10 to 60 minutes' immersion, usually 30 minutes, blood was drawn under liquid paraffin following the technic used by Goldschmidt and Light. Venous congestion was generally employed at the lower temperatures to facilitate bleeding. The tubes were coated with neutral potassium oxalate and sodium fluoride sufficient to make 0.3 and 0.03 per cent solutions respectively with the 20 cc. of blood usually drawn. All blood gas analyses were made with the long stem constant pressure Van Slyke apparatus. Duplicate analyses were only occasionally made. Some of the blood was drawn over mercury into a small tube and was used for determination of oxygen content and saturation. These analyses were made within a few hours, during which time the blood remained at room temperature (about 25°C.). As much as possible of the liquid paraffin was withdrawn from the remainder of the blood and replaced by hard paraffin. This blood was centrifugalised at room temperature, and the plasma was analysed for CO_2 content and the pH estimated colorimetrically. In this estimation the dilution was made as in the Hastings and Sendroy (1924) method; color matching was made either with their bicolor standards or with phosphate standards. These standards were frequently compared with other buffer solutions checked electrometrically; the blood values were also usually checked gasomet-

rically (Austin, Stadie and Robinson, 1925). A sample of the plasma was placed in a separating funnel and was rotated by hand while in contact with a mixture of expired air of known composition. The equilibration was made at room temperature (which was read by a delicate thermometer inserted into the funnel) and the gas mixture was renewed several times during the equilibration, which usually lasted at least 10 minutes. In addition the plasma was often allowed to stand in contact with the gas sample for 10 or more minutes between periods of rotation. On the sample so treated the pH level was estimated by colorimetric observations and by calculation from the Hasselbalch formula using the pH and solubility values given by Austin and Cullen (1926) for that temperature. As a rule the pH value so obtained was close to that of the blood as drawn. Any correction of the colorimetric value indicated by this comparison was then applied to all other colorimetric readings, regardless of the temperature at which they were made, since there is no evidence of any great variation of this "H correction" (Austin, Stadie and Robinson, 1925) with temperature. In every case this correction varied between +0.04 and −0.03;[1] the correction at room temperature may therefore be considered as approximately zero, considering the accuracy of the methods used.

Besides the colorimetric measurement (with gasometric check) made at room temperature, colorimetric matches were also made with the separated and diluted plasma warmed to 38°, and warmed or cooled to a temperature close to that estimated for the blood within the veins at the time of withdrawal. In making these matches the phosphate standards, when employed, were warmed or cooled, and a correction of −0.03 for a change of temperature from 20° to 38° was applied. When bicolor standards were used a correction of −0.13 for the effect of a change of temperature 20° to 38° on the color indicator, was applied (Hastings and Sendroy, 1924). In using both methods a linear relationship was assumed and at other temperatures corrections were made by intrapolation or extrapolation. The temperature of the blood sample was determined at the time readings were made by the method recommended by Hastings and Sendroy.

From the data so obtained, from those on tissue temperatures reported in the preceding paper and from venous temperatures reported by Harris and Marvin (1927), the probable gas tensions of the blood in vivo were calculated. In order to make comparisons with observed tissue gas tensions these temperatures were estimated on the basis of the probable vein temperatures within the water bath in the case of oxygen (midway between the two curves of figure 7 of the previous paper) and 5 minutes after withdrawal from the bath in the case of carbon dioxide. In this case the

[1] Occasionally these differences were exceeded, but only when the standards had not been recently checked.

data of figure 9 of the previous paper have been used to estimate the temperature after very hot or very cold baths. With baths of 25° to 34° the temperature after 5 minutes has been assumed to be the same as that within the bath, since the temperatures of the blood entering (at varying

TABLE 1

BATH TEMPERATURE	TISSUE TEMPERATURE	EFFECTIVE TISSUE TEMPERATURE FOR CO_2	PLASMA CO_2 CONTENT	pH 38°	pH AT TEMPERATURE FOR CO_2	CO_2 TENSION CALCULATED FROM BLOOD	TISSUE CO_2 TENSION	O_2 SATURATION	O_2 TENSION CALCULATED FROM BLOOD	TISSUE O_2 TENSION	ROOM TEMPERATURE
Subject S											
10.0	12.9	15.2	59.6*	7.38*	7.72*	13.9	20.5	90.2*	16.9	19.1	27
22.0	23.8	24.2	61.1*	7.39*	7.55*	24.2	25.8			14.5	27
34.1	34.5	34.5	60.1	(7.37)	(7.41)	38.0	31.7	84.6	46.1	63.0	18
37.2	37.2	36.5	60.3	7.35	7.37	42.9	40.0	85.4	55.0	62.9	25
39.6	38.7	37.4	65.2	7.35	7.36	46.8	45.0	71.3			26
40.8	39.9	37.6	59.6	7.39	7.40	40.0	39.3	90.2	77.7	68.0	27
42.0	41.0	38.0	61.1	7.39	7.39	42.1	46.7	90.0	83.4	72.5	27
Subject B											
10.0	12.4	15.2	61.0	7.41	7.75	13.4	27.6?	91.6	17.3	23.2	25
14.3	16.4	18.0	62.4	7.41	7.64	18.2	23.1			28.1	24
20.3	22.0	22.8	57.1	(7.45)	(7.65)	17.8	22.9				25
22.4	24.2	24.5	62.6	7.39	7.59	23.0	26.5	98.7	{76.4 / 42.2}	25.1	24
27.8	29.2	29.3	65.4	7.37	7.50	31.6	33.7			43.3	27
33.2	33.9	33.9	63.4	7.41	7.48	35.5	37.2	97.7	{97.4 / 69.4}	42.1	23
37.0	37.0	36.4	61.2	7.42	7.44	37.0	40.0	91.9	69.4	76.0	24
37.2	37.2	36.5	65.4	7.39	7.40	43.4	41.2	90.9	69.0	45.1	27
40.9	38.7	37.8	60.5	7.39	7.39	41.6	42.7	93.2	83.0	87.2	25
45.0	40.0	38.2	60.8	7.39	7.39	42.0	45.8	99.2	{202.4 / 100.0}	81.3	24

Values for pH in brackets represent colorimetric values without gasometric check.

Starred values for blood represent values determined from blood of opposite hand under different conditions.

When the oxygen saturation observed has been above 95 per cent in venous blood, its accuracy has been doubted and oxygen tensions have been calculated both from the observed saturation and from an assumed 95 per cent.

velocity) and of the surrounding air will tend to give a temporary balance at the existing temperature. Between 11° and 25°, and 34° and 42° a smooth curve was drawn to represent the combined effects of the bath and the early changes after withdrawal. The assumption has been made that the warming or cooling of the blood between withdrawal and centrif-

ugalisation caused no appreciable interchange between the corpuscles and the plasma (Joffe and Poulton, 1920), so that the pH estimated at any temperature for separated plasma has been assumed to be the same as that of true plasma at the same temperature.

RESULTS OBTAINED. The values obtained on blood drawn during experiments when tissue gas tensions were determined are given in table 1. In every case (except two in subject S) the blood samples were drawn from the hand subjected to the same conditions as the forearm from which the gases were withdrawn. In the two exceptions (starred in table) the blood was withdrawn from the opposite hand contained in hot water, owing to difficulty in withdrawing blood from constricted vessels. Our own evidence, as well as that of Goldschmidt and Light, shows that the oxygen saturation and CO_2 content are similar in bloods drawn at very high or very low temperatures, so that in these cases, probably no great error was introduced.

The carbon dioxide tension of the blood in vivo was calculated on the basis of the assumptions in regard to the effective temperature and blood constants already mentioned. That of oxygen was similarly calculated using the data for the probable temperature while the hand was in the bath, and for blood pH at this temperature; from these values $\log \frac{1}{K_0}$ for Hill's equation of hemoglobin dissociation was estimated. This estimation was done graphically by elaborating Stadie and Martin's data (1924); their assumption that $\log \frac{K_0}{K'_0} = \frac{17.100\ (T - T^1)}{4.6\ T\ T^1}$ at a pH of 7.4 has been used to allow intrapolation and extrapolation at a pH of 7.4 (see fig. 1). The slope of the lines has been taken from their diagram except that for 15° which did not quite pass through the point calculated by the formula; in this case the slope used is somewhat less steep than theirs, since it gives a little better agreement with observed oxygen tensions. The value of n has been taken at 2.39 (Stadie and Martin), and blood constants of the two subjects have been assumed not to differ greatly from those of Stadie. The actual oxygen tension was then calculated by Hill's equation.

It will be noted in the table that the CO_2 tensions calculated are somewhat lower than those observed in the tissues when the bath was at either very high or very low temperatures. The discrepancies may be due to errors in estimating the effective temperature but in any case they are not very great. The calculated oxygen tensions show greater variations from those observed in the tissues and are too high at the higher temperatures; the possibilities of error from wrong estimations of temperature, from wrong assumptions as to blood constants and from experimental errors in estimating oxygen saturation are so great, that the agreement is as good as could be expected. There is, therefore, no evidence that the

tensions in vivo are not simply dependent on the physical effects of temperature on blood constants and on a varying degree of venosity resulting from the velocity of blood flow relative to the rate of tissue metabolism. In order to establish more closely the relative values of these factors, the effects of temperature on the oxygen saturation, plasma CO_2 content and pH of the blood of subject B have been more fully investigated and the results are plotted in figure 2. The effect of temperature on the relation of pH to pOH in the blood is plotted in figure 3; in this case the points plotted represent the effective temperature for CO_2, i.e., the temperatures estimated as existing 5 minutes after removal from the water. The CO_2 and oxygen tensions calculated for varying bath temperatures from the smoothed curves drawn through the blood data are plotted in figure 4. It will be seen that the curves resemble those for tissue tensions reproduced in the previous paper, except that the curve for CO_2 is a little steeper and is not exactly a straight line, and that for O_2 has not the marked dip at 22° seen in the other figure. The values determined are insufficient in number to decide whether any real discrepancy is present.

Discussion. The blood data on subject B give a minimum oxygen saturation and maximum CO_2 plasma content at a bath temperature of 26° to 27°, a slightly higher temperature than that reported by Goldschmidt and Light (1925 b). The skin temperatures may have been similar, since the water was more violently stirred in our experiments.

The pH shift with temperature in vivo is mainly dependent on the changed physical constants of the blood and is only slightly affected by the

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Fig. 1. Relationship of $\log \frac{1}{K_0}$ to pH and temperature, plotted as an elaboration of Stadie and Martin's diagram. Values of $\log \frac{1}{K_0}$ are plotted from 1.9 to 3.8 as ordinates and the diagonal lines represent temperatures from 12° to 42°C. from below upwards. The 15° line is drawn through their observed point for a pH of 7.01, through the calculated point for a pH of 7.4 and above their observed point for a pH of 7.89.

Fig. 2. *Bath* temperatures are plotted as abscissae, and estimated blood pH in vivo within the bath, oxygen saturation and plasma CO_2 content as ordinates from above downwards. All data obtained in subject B.

Fig. 3. The pH, pOH and relationship of these to the neutral point of blood in vivo plotted against tissue temperature, following the nomogram of Austin and Cullen (1926). pH values are plotted as abscissae from 7.0 to 7.8 and pOH values as ordinates from 6.1 to 6.6. The diagonals running from N.E. to S.W. represent temperatures of 40°, 35°, 30°, 25°, 20°, 18° and 15° from left to right, and the other diagonals pH–pN 0.5, 0.6, 0.7 and 0.8 from left to right. All data obtained in subject B.

Fig. 4. *Bath* temperatures are plotted as abscissae and CO_2 and O_2 tensions calculated from the smoothed curves of figures 1, 2 and 3 as ordinates, for comparison with the curves representing observed tissue gas tensions in B reproduced in figures 4 and 6 of the preceding paper.

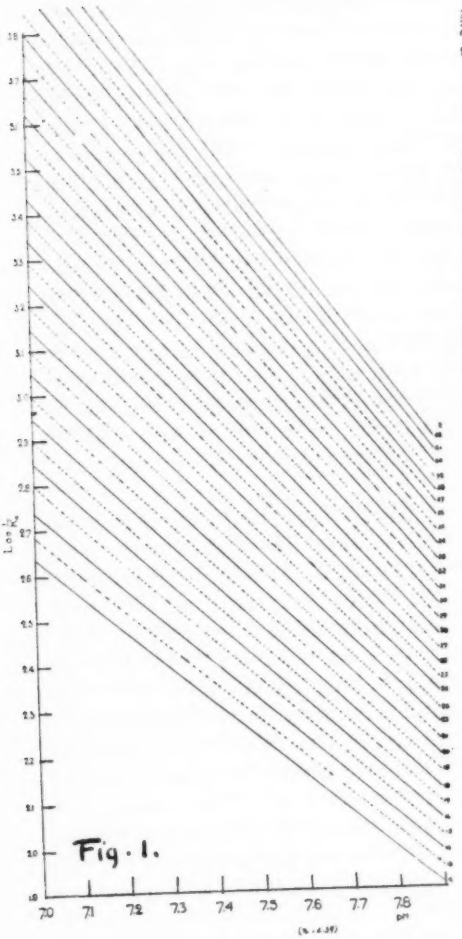


Fig. 1.

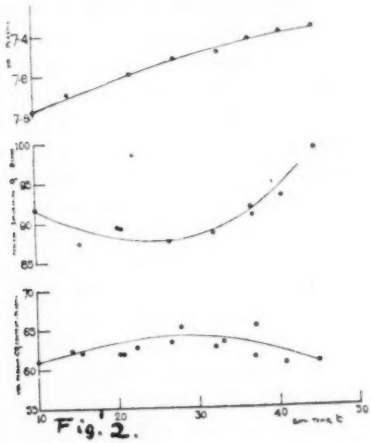


Fig. 2.

Fig 3.

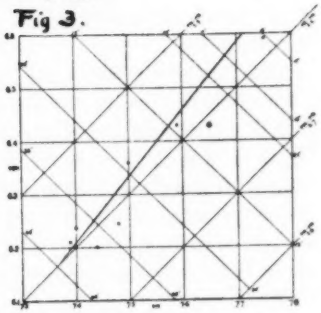
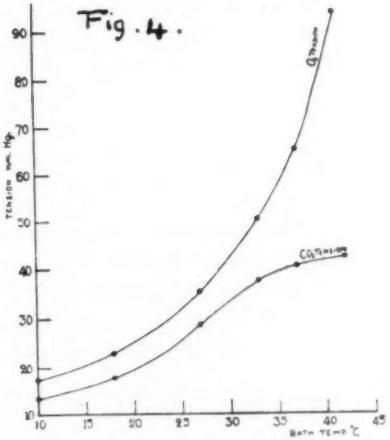


Fig. 4.



varying degree of venosity. For both subjects the estimated change in vivo is very similar to that observed in vitro with the separated plasma of these subjects, if the temperature is altered without loss of CO_2. The separated plasma gave an average increase of 0.24 pH unit on cooling from 38° to 20° in subject B and 0.23 in subject S. The change indicated for subject B in vivo by the line in figure 3 is 0.25. The discrepancies are within experimental errors. That plotted by Austin and Cullen (1926) for Stadie's whole blood is almost twice as great. On the other hand the data agree well with those of Stadie, Austin and Robinson (1926) on plasma. The pH value of the plasma had a mean value of 7.39 at 38° and a mean CO_2 content of 28 millimols and the change with temperature equalled that calculated by them for a β(Pr) value of about 2.0. Also if the data be plotted and curves relating $BHCO_3$ to pH similar to those used by Austin and Cullen (1926 table 6) be assumed, the data suggest a decrease of 0.016 pH units per 1°C. rise of temperature at constant $BHCO_3$ for true serum and 0.021 for separated serum, as compared with 0.017 to 0.023 reported by Stadie, Austin and Robinson for blood, true or separated serum.

It is, therefore, difficult to account for the discrepancy between the pH shift with temperature on Stadie's whole blood and that here reported; possibly the assumption that the temperature at which the blood is centrifugalised is of no importance is not true, but in that case nearly all the pH values for blood given in scientific literature would have systematic errors.[2] None the less, in case this should prove true, the room temperature at which centrifugalisation took place has been given in table 1. The presence of a systematic error is contraindicated by the general agreement between the CO_2 tensions calculated from the blood and those determined by air injection. Yet another check was provided by the results obtained in equilibrating the blood plasma with expired air. The average CO_2 tension in 9 such cases, where the pH of the equilibrated sample was the same ($\pm$0.03) as that of the blood as drawn, was 25.9 mm. for an average temperature of 25.7°. An effective tissue temperature of 25.7° should be found with a bath temperature of 23.5° and at this temperature the observed CO_2 tension in the tissues was 27.5 mm., giving a discrepancy of only 1.6 mm. The values at room temperature levels are therefore valid.

The agreement between calculated and observed oxygen tensions is also fairly good. At low temperatures the discrepancy between calculated and observed values is small; at the high temperatures the calculated values are usually too high, particularly where high oxygen saturations were observed. But a relatively small error in the saturation value at

[2] It is not possible for the curves relating pH to temperature for whole blood and separated plasma to differ, unless some interchange between corpuscles and plasma accompanies temperature changes.

these high saturations can make a difference of many millimeters (compare tensions calculated with 99.2 and 95 per cent saturation for a bath temperature of 45° in B) and the agreement between the observed tissue O_2 tensions and those calculated from average saturation and pH curves (fig. 4) is much better.

The thermodynamic studies of Brown and Hill (1923) suggested a value for n nearer 2.2 than 2.4, and 2.2 was accepted as the more probable by Barcroft and his collaborators (1922). Consequently the data reported on Stadie's blood have been recalculated using a value for n of 2.2, but with the K_0 values so obtained the calculated oxygen tensions of our bloods do not agree as well with observed tissue tensions. Recently Yabuki (1927) has reported data that indicate that the value of n increases with rise of temperature and decreases with increase in pH; it should therefore decrease considerably on cooling, where the pH value is also increased as in our experiments. Any values calculated on the assumption of a constant n must be mere rough approximations.

Our data have demonstrated that the changes observed in blood and tissues are in rough agreement, and that cooling a limb causes the blood to have a much lower oxygen tension, a lower CO_2 tension, and an increase in pH of about 0.014 unit for 1°C. fall of temperature. This change in pH is somewhat less than the increase in pOH, so that the cooled blood is nearer to the neutral point; the change is also less than that determined for the isoelectric point of blood proteins (0.017 to 0.023) by Stadie, Austin and Robinson (1925). On cooling, therefore, the value pI increases more than pH, and (pH − pI) is decreased; less base is therefore bound by protein and from this point of view the blood is more acid. If the chemical changes are concerned with the regulation of the blood flow in the vessels, either the CO_2 tension or hydrogen ion concentration would appear to be concerned. There has been no evidence of any tendency to maintain a constant pH − pI value, such as was observed in cold-blooded animals by Austin, Sunderman and Camack (1927).

CONCLUSIONS

1. The oxygen saturation, plasma CO_2 content and plasma pH have been measured on blood obtained from the hand veins after immersion of the hand and forearm in baths at different temperatures in the same subjects used for observations on tissue gas tensions. The gas tensions calculated from the blood data agree fairly well with those observed in the tissues.

2. The pH values of the separated blood plasma have been estimated at 38° and at the temperature at which the blood probably was before it was drawn. The change in pH of the separated plasma in vitro with temperature is almost the same as that estimated (from separated plasma data) for the blood in vivo. The effect of variations in venosity is small compared with the effect of changes in temperature on the physical constants.

3. Venous blood is estimated to have a pH value increasing by 0.014 and a pOH value increasing by 0.018 for each degree fall of temperature.

We would like to take this opportunity of expressing our thanks to Doctors Goldschmidt and Austin for valuable advice both on technical details and on general principles, and to Dr. C. G. Johnston for checking electrometrically some of the phosphate standards used.

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IMPROVEMENTS OF THE WELCKER METHOD FOR THE DETERMINATION OF THE TOTAL BLOOD VOLUME—THE RELATION OF THE BLOOD VOLUME TO BODY WEIGHT AND SURFACE OF THE PIGEON

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The determination of the total blood volume of a small animal, such as the pigeon, necessitates the use of a method that is both accurate and easily carried out. The vital red method of Keith et al. (1915) is impracticable for this purpose, since the withdrawal of sufficient blood for the standard and the subsequent injection of the dye solution would result in a 10 to 25 per cent alteration of the entire blood volume with each procedure. The carbon monoxide method, as applied to rabbits by Boycott and Douglas (1909) and to rats by Scott and Barcroft (1924), likewise did not appear advisable, primarily because it gives rather variable results in the hands of different workers (Lamson and Nagayama, 1920; Erlanger, 1921). It also requires considerable technical skill.

A more promising procedure appeared to be a simple modification of the Welcker method, involving the sacrifice of the animals and subsequent collection of the blood by hemorrhage and by washing out the vascular system. The efficiency of such a method obviously depends on the degree of accuracy obtainable in determining the whole blood content of the washout fluid. This point was checked by diluting a known amount of blood to various degrees with 0.85 per cent NaCl, as shown in table 1, and determining after each dilution, by means of the cell count method of Malassez (1874), the difference between the observed and actual blood present. The error was quite small and it may be observed that with a dilution of 1 to 600, the error was somewhat greater with the carbon monoxide hemoglobin determination than by the cell count method.

Malassez further modified Welcker's method by substituting an artificial serum containing gum arabic, NaCl, Na_2SO_4 and anticoagulants for the water, which had been used by Welcker. This artificial serum would seem unnecessarily complicated and even disadvantageous, because the perfusion of the vascular system would be slower and less complete than should be obtainable with an isotonic perfusate of lower viscosity. This

point has been verified by Boycott and Douglas, who perfused the vessels, after hemorrhage, with 0.8 per cent NaCl in 1 per cent sodium citrate.

DESCRIPTION OF METHOD. The following method, which incorporates certain features of each of the older methods, was found to give the most favorable combination of convenience and accuracy.

The normal red cell count and specific gravity of the whole blood were determined (the latter by the Hammerschlag method), taking the mean of at least three readings, which were obtained either separately or at daily intervals. The blood samples required for these purposes were always taken from the same marginal wing vein of pigeons. The animal was then decapitated, and the total blood obtainable by hemorrhage was collected and measured. A cannula was placed in a jugular vein toward the heart, the carcass suspended by the feet, and two to three liters of 0.85 per cent NaCl perfused by hydrostatic pressure of 140 to 150 cm. until the outflow was colorless. Frequent massaging with occasional

TABLE 1

Error of determination of dilution of rabbit's blood by cell count and by carbon monoxide hemoglobin

		ERROR PER CENT
Normal red cell count	6,416,000	
Hemoglobin	95 per cent	
Volume sample drawn	5.0 cc.	
Volume determined by cell count; dilution 1:300	4.92 cc.	−1.6
Volume determined by cell count; dilution 1:400	5.06 cc.	+1.2
Volume determined by cell count; dilution 1:600	4.84 cc.	−3.2
Volume determined by CO Hb; dilution 1:600	4.675 cc.	−6.5

interruption of perfusion facilitated a more rapid and complete washing. No anticoagulant was found necessary. The outflow from the cut surface was collected in a glass container and a homogeneous suspension maintained throughout the period of perfusion by means of an electrically driven stirrer.

On completion of the washing of the vessels, the red cell count of the blood suspension, without further dilution, was determined repeatedly until two consecutive readings checked within 2 per cent. The actual blood content of the suspension in cubic centimeters was then determined by dividing the total volume of the suspension in cubic centimeters by the dilution factor, namely:

$$\frac{\text{normal red cell count}}{\text{red cell count of suspension}}$$

The total blood volume in cubic centimeters is represented by the sum of the volumes obtained by hemorrhage and perfusion respectively. The relation between the total blood and the body weight may be obtained by dividing the blood weight (i.e., volume in cubic centimeters times specific gravity) by the body weight in grams. These calculations are illustrated by the following example:

Pigeon 25

Body weight in grams = 337
Normal red cell count = 3,860,000 per cmm.
Specific gravity (blood) = 1.066
Blood recovered by hemorrhage = 9.7 ccm.
Volume of perfusate (blood) suspension = 2350 ccm.
Cell count of perfusate = 397 × factor 40 to give cells in 1 cmm. = 15,880

$$\text{Dilution of blood in suspension} = \frac{\text{normal count (3,860,000)}}{\text{perfusate count (15,880)}} = 243.07$$

$$\text{Actual blood content of perfusate} = \frac{\text{perfusate volume (2350 cc.)}}{\text{blood dilution in perfusate (243.1)}} =$$

9.68 ccm.
Total blood volume = hemorrhage (9.7 cc.) + perfusion (9.68 cc.) = 19.38 ccm.

$$\text{Blood weight per cent of body weight} = \frac{\text{Total blood in ccm. (19.38)} \times \text{specific gravity (1.066)}}{\text{Body weight in grams (337)}} = 6.10 \text{ per cent}$$

The operations are very simple so that after a little practice the operator should be able to determine the blood volumes of eight to twelve animals in a day. For purpose of comparison the blood volumes obtainable by the above outlined procedures and by the Gscheidlin (1873) modification of Welcker's method were ascertained on each of a series of pigeons.

In order to carry out the double procedure, the pigeon was bled into a volumetric flask containing a known volume of a 0.4 per cent solution of aqua ammoniae in distilled water. On making up to volume, the volume of blood was computed by subtraction. This sample then served as the standard for the colorimetric determination of volume. The authors' procedures were then completed as outlined.

The determination of the blood content of the suspension by the Gscheidlin method necessitated laking of the red cells. This was accomplished by adding concentrated ammonia to the suspension to make a 0.4 per cent solution and further dilution with 0.4 per cent of aqua ammoniae in water. A portion of the standard was then diluted until the comparison between the standard and the laked suspension was close. Both samples were then saturated with carbon monoxide and the actual depth of color in the suspension determined colorimetrically. The whole blood content of the laked suspension was represented by the blood equivalent of 1 ccm.

$$\text{of the standard} \times \frac{\text{standard (colorimeter) reading}}{\text{suspension (colorimeter) reading}} \times \text{volume of suspension}$$

in cubic centimeters.

The bones and soft tissues, with the exception of the feathers, skin, head, feet and gastro-intestinal tract, were minced and extracted with water for eighteen hours, to obtain an index of the blood remaining in the tissues after hemorrhage and perfusion.

The blood volume obtainable by the cell count method, i.e., hemorrhage and perfusion, does not show any marked difference from that obtainable by the Gscheidlin method. The results obtainable by the former procedures are somewhat higher (0.3 ccm. of blood per 100 grams body weight), than are shown by the latter, but very possibly fewer errors are encountered by the cell count method. The water extraction of the bones and muscles after hemorrhage and washing out of the blood vessels, apparently yields 1.2 to 2.38 ccm., median, 1.5 ccm., of blood as determined colorimetrically. This value is approximately $\frac{1}{13}$ or 7.5 per cent of the entire blood volume, almost exactly that determined by similar methods by Boycott and Douglass (1909), for rabbit tissues. Due to obvious sources of error in such extracts, i.e., turbidity and myohemoglobin content the results are rather misleading and probably too high. We prefer, therefore, to omit the time consuming tissue extraction altogether and consider the loss as negligible. Suter and Jaquet (1897) also concluded that the colorimetric error due to turbidity was greater than that due to an incomplete washout of the vessels.

The average blood volume of normal pigeons. The application of the authors' modification of Welcker's method is illustrated by the blood volume data of the series of forty-two birds, presented in table 2. This shows that the data are quite uniform. The median blood weight in grams, in terms of per cent body weight has the value of 6.56 per cent.

This value is almost $\frac{1}{5}$ higher than the average of Welcker's figures. However, the results are more uniform, i.e., show a smaller variation from the mean, than corresponding figures obtained either by Welcker, or with the subsequent modifications of the latter's method by Gscheidlin (1873) or Malassez (1874). The range of the blood weight, shown in table 2, was from 5.62 to 8.1 per cent. These divergences from the mean however were exceptional as 85 per cent of all observations were within ± 7.5 per cent of the mean.

The deduction of the feather weights from the gross body weights increased the pigeon blood volume, in terms of body weight approximately $\frac{1}{10}$. The resulting figure corresponds closely to data obtained for man by the hemorrhage and washout method (Erlanger, 1921).

RELATION OF BLOOD WEIGHT AND VOLUME TO BODY WEIGHT AND SURFACE. When the individual blood weights are plotted against the

TABLE 2

Blood-volume of pigeons obtained by the modified Welcker's method

SERIAL NUMBER	WEIGHT	RED CORPUSCLE COUNT PER CMM.	SPECIFIC GRAVITY OF WHOLE BLOOD	TOTAL BLOOD VOLUME (CCM.)	BLOOD WEIGHT PER CENT OF BODY WEIGHT
	grams			*cc.*	
1	275	4,160,000	1068	15.70	6.07
2	281	4,056,000	1065	16.80	6.37
3	290	3,660,000	1064	21.80	7.99
4	293	4,076,000	1063	19.03	6.90
5	296	4,080,000	1057	22.80	8.10
6	300	3,824,000	1069	15.75	5.62
7	300	3,816,000	1068	18.20	6.48
8	307	4,092,000	1061	21.09	7.29
9	325	3,856,000	1062	19.45	6.36
10	328	3,960,000	1062	18.20	6.00
11	337	3,860,000	1066	19.38	6.10
12	340	4,060,000	1067	19.22	6.04
13	341	4,000,000	1054	18.10	6.00
14	342	4,264,000	1067	22.70	8.10
15	349	4,008,000	1071	21.34	6.55
16	352	3,836,000	1062	24.20	7.30
17	356	3,600,000	1059	20.04	6.00
18	368	4,152,000	1063	20.40	5.89
19	370	4,320,000	1063	20.80	6.00
20	408	3,928,000	1066	26.60	6.95
21	348	4,064,000	1067	19.80	6.07
22	324	4,256,000	1068	26.20	8.63
23	291	4,224,000	1075	18.20	6.72
24	261	3,796,000	1061	18.84	7.64
25	277	3,880,000	1059	18.90	7.28
26	312	4,160,000	1068	20.43	7.00
27	337	3,848,000	1059	22.96	7.21
28	312	3,936,000	1066	20.52	7.01
29	289	3,808,000	1066	15.94	5.89
30	333	4,408,000	1066	20.00	6.39
31	347	4,208,000	1060	22.60	6.88
32	263	3,968,000	1064	17.60	7.10
33	337	3,440,000	1051	21.36	6.66
34	295	3,816,000	1062	19.80	7.08
35	360	3,780,000	1060	22.40	6.58
36	359	4,264,000	1068	22.00	6.54
37	325	4,368,000	1063	20.57	6.72
38	341	4,344,000	1066	20.79	6.50
39	320	4,064,000	1063	20.20	6.74
40	290	4,128,000	1064	17.40	6.39
41	301	3,756,000	1058	17.60	6.19
42	361	3,944,000	1068	19.48	5.75
Median.....	325	4,004,000	1064	20.02	6.565

respective body weights, as illustrated in figure 1, the direction of the traveling median suggests that the percentile blood weight or volume decreases inversely to body weight increase. This inverse relation between the blood weight and body weight is not a function of the age of the animals, for both the large and small animals were adult as shown by the constant body weight level during the six- to seven-week preliminary feeding period.

When the total blood volumes in cubic centimeters of a series of normal pigeons are plotted against the net or featherless body weights of the birds (fig. 2), it may be observed that the dots distribute themselves almost equally on either side of and their median corresponds to the solid line which represents the theoretical blood volume of the same series calculated

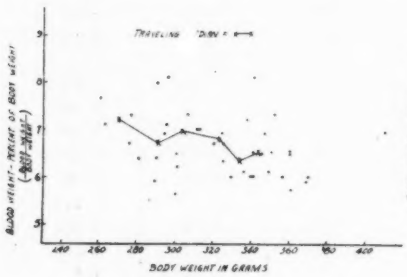

Fig. 1

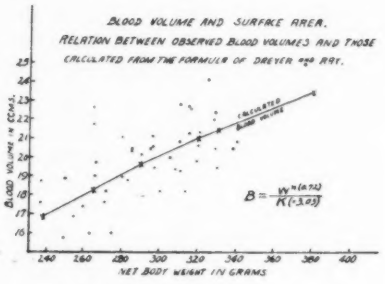
BLOOD VOLUME AND SURFACE AREA.
RELATION BETWEEN OBSERVED BLOOD VOLUMES AND THOSE CALCULATED FROM THE FORMULA OF DREYER AND RAY.
NET BODY WEIGHT IN GRAMS

Fig. 2

from the body surface by the Dreyer-Ray (1912) formula. It is interesting to note that the close correspondence which these authors have shown to exist between blood volume and body surface rather than body weight of rabbits, likewise holds true for pigeons.

Specific gravity of the whole blood of normal pigeons. The specific gravity of the whole blood of normal pigeons is relatively uniform. The extreme readings noted (see table 2) i.e., 1051 and 1075, do not represent the true range which should be 1054 to 1069 and as a rule the variation from the median value of 1064 is within ± 6 points. The significance of these variations in the specific gravity of the whole blood will be discussed in a separate paper.

SUMMARY

The blood volumes of small animals can be determined satisfactorily by combining certain of the modifications of Welcker's method, involving a determination of the normal red cell count, complete hemorrhage, and washing out of the vascular system with 0.85 per cent NaCl, with subsequent calculation of the blood content of the perfusate by means of its cell count and degree of dilution.

The method is quite as accurate as any which has been described, and has the further advantages of simplicity and economy of time.

Normal pigeons have a total blood weight equivalent to 6.5 per cent of the gross body weight, but total blood volume in cubic centimeters is more closely associated with body surface than with body weight.

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THE INFLUENCE OF THE DAILY ORAL ADMINISTRATION OF MAGNESIUM SULPHATE OR LACTOSE ON THE BLOOD VOLUMES OF NORMAL, BERI BERI AND FASTING PIGEONS

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The administration of magnesium sulphate, lactose or mineral oil has been shown to prevent and remove the anemia of rice disease in a large percentage of the pigeons studied (Barlow, 1927). The beneficial effects of these agents were tentatively attributed to their cathartic action, with a consequent limitation of bacteriemia. There was the possibility, however, that blood concentration might be a factor in the maintenance of the blood counts of the treated birds, although at the time the observations were taken, i.e., 22 to 26 hours after the last administration of any cathartic, the dehydration factor was considered as probably negligible (Underhill and Errico, 1922). However, it seemed safer to settle the matter directly. In the present paper a comparison is therefore made between the changes in body weights, red cell counts and total blood volumes of three groups of pigeons which had been placed, respectively; on a normal diet, polished rice diet and complete fast, each with and without the daily administration of magnesium sulphate or lactose.

METHODS. Adult birds ranging from 280 to 408 grams in weight were given a normal diet consisting of mixed whole grain and grit for four weeks. Subsequently, the control observations, including the red cell count, hemoglobin per cent, blood specific gravity and body weight were obtained on each pigeon at seven day intervals over a three week period. The cell counts were made with the Leitz—U. S. Bureau of Standards Chamber. The hemoglobin was estimated by the Newcomer method, the specific gravity of the blood by the Hammerschlag method.

The pigeons were then divided into nine groups, according to the diet and treatment received, as shown in table 1, and the observations continued at weekly intervals on the birds receiving a normal or mixed whole grain (N), or polished rice (R) diets, and on the seventh and tenth days in the fasting (F) experiments (see table 1). In those experiments in which either magnesium (M) or lactose (L) was administered in addition to the diet, (see column 4, table 1) all readings were made 22 to 26 hours after the last dose

had been given. The magnesium sulphate was administered in gelatin capsules and the lactose as a saturated watery solution by tube. At the conclusion of the period of study, as shown in table 1, the total blood volume of each bird was determined by the modified Welcker method as explained in accompanying article.

CHANGES OF WEIGHT, RED CELL COUNT AND BLOOD VOLUME OF PIGEONS RECEIVING A POLISHED RICE DIET (fig. 1, *R-O*) OR IN FASTING (fig. 1, *F-O*). The administration of polished rice over a three-week period results in loss of weight, *A*, depression of the red count, *B*, decrease in total or absolute red cells, *C*, (i.e., red cells per cubic centimeter times the blood volume in cubic centimeter) as well as per kilogram *E*, decrease in the specific gravity of the whole blood, and diminution of the total blood weight or absolute blood volume per body weight, *D*, and per kilogram, *F*.

The red blood cell count falls, although not quite at the same rate as the

TABLE 1

GROUP LETTER	NUMBER OF PIGEONS PER SERIES	DIET	TREATMENT PER BIRD	PERIOD OF STUDY
				days
N-O	20	Normal	None	21
N-M	5	Normal	$MgSO_4$—1 gram daily	21
N-L	5	Normal	Lactose—2 grams daily	21
R-O	6	Polished rice	None	21
R-M	6	Polished rice	$MgSO_4$—1 gram daily	21
R-L	8	Polished rice	Lactose—2 grams daily	21
F-O	7	Fast with access to water	None	10
F-M	6	Fast with access to water	$MgSO_4$—1 gram. daily	10
F-L	6	Fast with access to water	Lactose—2 grams daily	10

drop in body weight. This divergence might be explained on the basis of hydremia, greater destruction of red cells or both. The blood volume however decreases at a smaller rate than the body weight (*A*, *C*, *F*), so that there is a relative[1] plethora. The absolute blood count decreases at the same or a slightly greater rate than the body weight (*B*, *E*), so that the relative plethora is actually hydremic.

The whole process therefore in rice disease, as well as in fasting is a diminution of blood volume as well as body weight. The corpuscles decrease more rapidly than the serum and less rapidly than the body weight. This latter relationship results in hydremia and this in turn in a (relative) hydremic plethora. The corpuscle decrease shows a relative as well as an absolute anemia.

[1] The "relative," throughout this paper, means relative to the loss of body weight.

This tendency for the development of hydremia in rice disease is in harmony with the observations of Krause (1922), who noted an increased

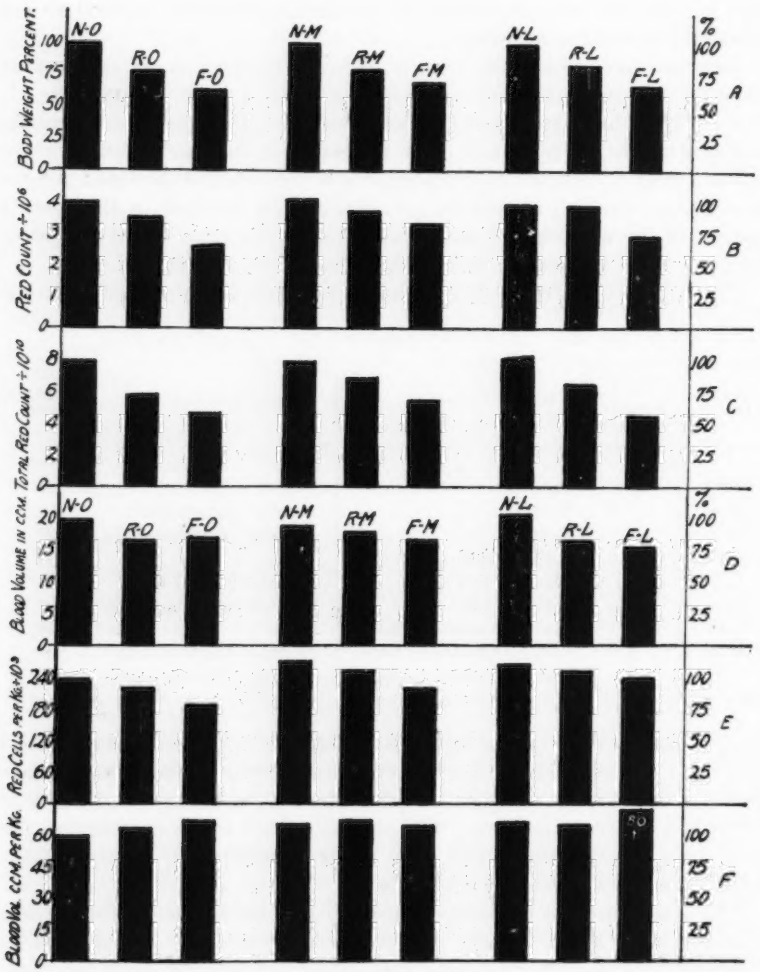

Fig. 1

water content of the blood, as well as of various tissues under similar conditions. The blood volume changes observed by the authors with fasting pigeons are in contrast to those of Panum (1864), who stated that the

normal relationship between blood volume and body weight was maintained in young pups irrespective of starvation. The hemorrhage and washout method used by Panum, may have resulted in an incomplete blood recovery. Terrioine et al. (1922), however, also observed a definite increase in the water content of bird tissues during the course of starvation.

THE INFLUENCE OF THE DAILY ADMINISTRATION OF CATHARTICS ON THE BLOOD VOLUMES OF PIGEONS. *Normal diet.* The red cell count, specific gravity of the whole blood and total blood volumes of a series of pigeons receiving orally 1 gram of magnesium sulphate or 2 grams of lactose daily over a twenty-one day period, in addition to a normal diet (fig. 1, *N-M*, *N-L*) did not show significant differences from their controls (fig. 1, *N-O*) at the time the observations were taken. The slight variations in *D*, *E* and *F*, are explainable by differences in the original "normal" weights of the birds of the three groups.

THE INFLUENCE OF THE DAILY ADMINISTRATION OF CATHARTICS ON THE BLOOD VOLUME OF PIGEONS IN FASTING OR IN RICE DISEASE. The identical treatment of rice or fasting birds with magnesium sulphate (fig. 1, *R-M*, *F-M*) or lactose (fig. 1, *R-L*, *F-L*) did not significantly alter the progress of inanition, i.e., weight loss, *A*, usually observed under such conditions in the absence of these cathartics (see fig. 1, *R-O*, *F-O*).

In both rice disease and fasting, the oral administration of magnesium or lactose tends to limit the fall in the red cell count usually observed in untreated birds, confirming previous work (Barlow, 1927). The total corpuscle count (*C* and *E*) also diminishes less than under similar dietary conditions, showing that magnesium sulphate or lactose actually diminish the usual rate of red cell destruction in inanition.

The blood volumes of the treated birds (*D* and *E*) do not differ significantly from those of the untreated series or control series (*R-O*, *F-O*). Magnesium sulphate or lactose apparently does not alter or counteract the hydremic plethora which develops in either fasting or rice disease.

SUMMARY AND CONCLUSIONS

1. The loss of body weight in the development of inanition as seen in pigeons, in rice disease or in complete fasting is accompanied by diminution of the red cell count and total blood volume. The red corpuscles tend to decrease somewhat more than the body weight but the ratio of blood weight increases, indicating the development of a relative hydremic plethora.

2. The anti-anemic effect of magnesium sulphate or lactose during the course of inanition is not due to concentration of the blood but to interference with blood destruction.

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A HORMONE MECHANISM FOR GALL-BLADDER CONTRACTION AND EVACUATION

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The numerous reports in the literature showing the marked efficacy of fats, egg-yolk and meat protein in emptying the gall bladder convinced us that the effect of "secretin" on the evacuation and motor activity of the gall bladder should be studied, since it is well known that these substances stimulate the pancreas, and that "secretin" (very impure solutions) promotes the formation of bile. With this objective in mind, we have performed several series of experiments, the results of which prove, we believe, that a hormone mechanism is concerned in gall-bladder contraction and evacuation.

For some time Ivy, Kloster and Lueth (1927) have been working on the purification of "secretin" for the purpose of perfecting a pancreatic function test for man. In our work we have used the preparations prepared for us by them. The solutions furnished us were "highly purified," were vasodilatin-free, and have had no objective deleterious action when injected intravenously in succession in anesthetized or unanesthetized animals.

In a search of the literature it has been found that several investigators have attempted to determine whether or not a humoral mechanism is concerned in gall bladder evacuation. Boyden (1926a) found that the transfusion of blood of fed or starved cats caused a partial evacuation of the gall bladder. He does not state that he tested the compatibility of the blood of the cats used. He interpreted the results of his experiments on this phase of the gall bladder question as meaning that the gall bladder is sensitive to bodily conditions which effect changes in the circulating blood. Whitaker (1926) injected doses of "secretin" prepared by the Bayliss-Starling method with negative results. Copher and Illingworth (1928) injected "secretin" with negative results; but they do not state the method by which the "secretin" used by them was prepared. Brugsch and Horsters (1926) placed a condom balloon in the gall bladder of dogs and found that 10 cc. of a preparation of Dale and Laidlaw (1912–13) "secretin" caused a contraction and rhythmic changes of the gall bladder, and "hypo-

physin" relaxed it. They observed that histamine (10 mgm.) caused the gall bladder to contract. They concluded that histamine and "secretin" act alike, the latter being the physiological agent. They did not make a simultaneous blood pressure tracing to rule out vaso-dilatation as being a factor, which we know is a factor.

EXPERIMENTS. *On cats without cystic duct clamped.* The cat was barbitalized, the common bile duct was cannulated, and a cannula was placed into the gall bladder through a small opening in the fundus. The cannula in the gall bladder was connected to a glass tube, which served as a manometer. The glass tube was connected to a recording tambour. A simultaneous carotid blood-pressure tracing was made. Four such experiments were performed. Within one minute after the intravenous injection of the "purified secretin" (2 mgm. of solid material), the intra-gall-bladder pressure was increased, and steadily increased until it amounted to from 2 to 3.5 cm. of bile pressure. About four minutes after the injection the bile flow was augmented. The pressure started to decline in from ½ to 1 hour in these experiments. The blood pressure, respiration and heart rate were unaffected by the injection. Normal saline controls proved negative.

On cats with cystic duct clamped. To avoid entirely the possibility that the increase in gall bladder pressure might be due to an inflow of bile, which was not likely, we clamped the cystic duct. Three experiments of this kind were performed. In all experiments the gall-bladder pressure was raised on the injection of the "secretin," but not to the extent that occurred in the preceding experiments. This difference, we believe, was due to some interference with the blood supply of the gall bladder, which is very difficult to avoid in the cat on ligating or clamping the cystic duct.

Atropine and histamine in cats. It was found that the "secretin" preparation caused as much contraction after the injection of 1 mgm. of atropine as it did before the injection of atropine. Two such experiments were performed.

The injection of 0.5 mgm. of histamine intravenously in the cat caused a decided fall in blood pressure without changing the intra-gall-bladder pressure. This effect is different from that which occurred in the dog, which will be shown later in this paper.

On dogs with the cystic duct clamped. Dogs were anesthetized with barbital (0.200 gram per kilo body weight) or with "light" ether. The pancreatic duct was cannulated, the cystic duct was clamped above the opening of the right hepatic duct, and the fundus of the gall bladder was cannulated. The cannula was connected to a glass tube which served as a manometer, which in turn was connected to a recording tambour. The carotid was connected to a recording blood pressure manometer. In many experiments the cystic artery was included in the clamp, it being impossible to prevent this without anatomical dissection, which we wished to avoid.

When the "highly purified secretin" preparation was injected intravenously (3 mgm. of solid material) the intra-gall-bladder pressure began to rise in from one to two minutes, even before the pancreas began to secrete. The rise of pressure from a single injection has varied in over eighty experiments from 1.0 cm. of bile pressure to 11.5 cm. of bile pressure. We have found only one dog out of more than eighty which has failed to respond. Why, we do not know. The injection, as a rule, causes no change in blood pressure, heart rate or respiration. Occasionally a 5 or 10 mm. rise followed by a fall of 5 or 10 mm. in blood pressure occurs, which we believe is due to the handling of the femoral vein into which all of our injections have been made. Sometimes a 5 or 10 mm. fall will occur which may persist from one to ten minutes (figs. 1, 2 and 3).

We believe that one cause of the variation in the amount of increase in intra-gall-bladder pressure which occurs is due to the inclusion of the cystic artery in the clamp. The greatest rises have usually occurred in those animals in which it was possible to clamp the duct without at the same time clamping the cystic artery.

In some animals in which the tonic contraction amounts to only a 3 cm. rise in pressure, rhythmic contractions amounting to as much as 2.5 cm. of bile pressure occur superimposed on the tonic contraction. As a rule, when the tonic contraction is quite marked the rhythmic contractions disappear at the height of the tonic contraction.

Our experiments show that there is an optimal initial pressure necessary for an optimal response to the injection. This optimal pressure is approximately 6 cm. of bile pressure. In this connection we have found that if, due to the spilling of some bile during the insertion of the cannula into the fundus of the gall bladder, the bile does not rise in the cannula several centimeters above the gall bladder, a response to the injection may not occur; but if some normal saline (37°C.) is placed in the cannula so that it rises in the tube for 5 or 7 cm., then the injection results in a decided contraction.

The period of increased gall-bladder pressure due to one injection lasts from 10 to 60 minutes, the average being about 15 minutes. If a tonus rhythm is not being manifested by the gall bladder prior to the first injection, it usually appears during the period of the relaxation of the tonic contraction and frequently persists for the remainder of the experiment, which in the process of assaying various preparations for further purification may last as long as six to eight hours.

If the animal has been fed prior to barbitalization, or if the gall bladder has been "energized" by a meal, the injection of the "secretin" preparation causes only a slight response. This is analogous to our experience that the gall bladder frequently fails to respond after it has been caused to contract some ten or twelve times within three or four hours. The maximum rise

due to a single injection was 11.5 cm. of bile, and the maximum rise due to a series of injections was 22.5 cm. of bile, six injections being made at five or ten minute intervals. We have observed no difference in the gall-bladder response of male and female dogs.

Bile and bile salts. The intravenous injection of dilute bile and one per cent solutions of sodium glycocholate and taurocholate failed to cause gall bladder contraction.

Atropine and histamine in dogs. Even 10 mgm. of atropine sulphate will not prevent the rise in gall-bladder pressure when the "secretin" preparation is injected (fig. 5).

Histamine causes in the dog a contraction of the gall bladder which is a mirror image of the fall in blood pressure. Every agent that we have injected which causes a fall in blood pressure has caused a contraction of the gall bladder (fig. 3).

Deep anesthesia. Moderate and deep ether anesthesia as a rule prevents a contraction from occurring. Deep barbital anesthesia, we believe, decreases the response, especially if the blood pressure is low.

On unanesthetized dogs with hepatic ducts tied and common duct cannulated. Under morphine-ether anesthesia, with aseptic technique, the hepatic ducts of two dogs were tied, and a cannula was inserted into the common bile duct before it entered the duodenal wall. The cannula was connected to the exterior with a rubber tube, which was clamped to hold the bile in the gall bladder. The dogs were trained to lie quietly. From twelve to twenty-four hours later the rubber tube was connected to a glass tube, serving as a manometer, which in turn was connected to a recording tambour. A single injection of the "secretin" preparation caused the pressure to rise from 20 to 30 mm. of bile, where it was maintained for from 30 to 60 minutes, after which it gradually returned to normal. In one of these dogs we injected one dose every four minutes for five injections. A step-like rise in pressure resulted. The original pressure prior to the first injection was 18.0 cm. of bile, and after the last injection it gradually rose to 24.0 cm. of bile pressure. After about one-half to one hour it slowly returned to normal (fig. 6).

On unanesthetized dogs with the cystic duct cannulated. Under morphine-ether anesthesia, with aseptic technique the cystic duct was cannulated above the opening of the right hepatic duct in such a manner as not to spill any of the bladder bile. The cannula was connected with the outside by a rubber tube, which was clamped to prevent the loss of bile. The following day, the clamp was removed and the rubber tube was connected with a manometer and a recording tambour. Two animals were used. In one of the animals, the usual contraction of the gall bladder on the injection of "secretin" was observed, as described in the preceding paragraph. In the other animal, it was found that fluid would pass into the gall bladder,

but the cannula was so occluded that changes in intra-gall-bladder pressure could not be recorded. We decided to inject the "secretin" preparation. Following the first injection nothing occurred; after the second injection the animal salivated (denoting nausea); after the third injection, the animal vomited and could not be kept quiet. Since this was the only time that we had elicited vomiting by injecting our material, and since the animal reacted as animals frequently do when a balloon is placed in the gall bladder and distended, we concluded that the contraction of the gall bladder with the cystic duct occluded caused the distress.

Evacuation of lipiodol (iodized oil) from the gall bladder in the unanesthetized dog by successive injections. Under morphine-ether anesthesia and with aseptic technique, the gall bladder (ten dogs) was filled with lipiodol (iodized oil) after the removal of an aliquot portion of bile (from 15 to 20 cc. usually). The dog was trained to lie quietly. From eighteen to twenty-four hours later, the animal not being fed, serial roentgenograms (from 2 to 10 minutes apart) were made, one dog's dose of the "secretin" preparation (a dose known to cause gall-bladder contraction) being injected intravenously every ten minutes for one hour. A study of the roentgenograms and autopsy showed that in three of the ten dogs, all but about 3 cc. of the lipiodol had been evacuated; in three, about two-thirds of the lipiodol had been evacuated; and in three, about one-half had been evacuated. In one, only a small quantity was evacuated. This animal was given, three hours later, a meal of cream (200 cc.), egg-yolks (6) and some milk. It was found that this meal caused no evacuation. The next morning a radiological examination and a picture showed a dense shadow, the lipiodol, and a line about 2 cm. away from the lipiodol shadow, which we interpreted as the gall bladder wall. We believed that fresh bile had entered the gall bladder during the night. Three injections of the "secretin" preparation were then given with the result that the outer line was caused to move in and make contact with the lipiodol shadow. We believe that this denoted that the fresh bile had been evacuated. Autopsy revealed that the lipiodol mass was almost gelatinous in consistency, and we believe so viscous that it could not be evacuated by the gall bladder. Similar gall bladder evacuation can be accomplished in dogs under light barbital anesthesia (figs. 7, 8 and 9).

It should be pointed out that lipiodol is more viscous than normal gall-bladder bile, and hence is more difficult to evacuate from the gall bladder.

An occasional roentgenogram from some of the dogs shows that the hepatic ducts are injected with lipiodol for from 0.5 to 3 cm. This occurred more frequently in the case of the right hepatic duct than in the others. We believe that this injection of the hepatic ducts is most probably due to increased pressure on the intramural portion of the common bile duct exerted by the duodenum, preventing the outflow of the lipiodol into the duodenum.

The injections of the "secretin" preparation had no objective nor detectable effects on the animal.

When we carefully observed the gall bladder under the fluoroscope, we could see it change its contour. We only observed definite changes in the half adjacent to the neck of the gall bladder. If the dome of the gall bladder contracted, we did not see it.

On contraction of the gall bladder caused by hydrochloric acid in the duodenum. Ten experiments were performed in which the cystic duct was clamped and the gall bladder cannulated under barbital anesthesia. We introduced by means of a needle and syringe, from 10 to 40 cc. of N/10 HCl into the duodenum and observed contraction of the gall bladder in every instance. The minimal quantity with which we were able to elicit a response was 15 cc., but we believe that under more normal experimental conditions less acid would be necessary (figs. 9 and 10).

The latent period of contraction was in most instances about two minutes; in a few instances about five minutes; whereas, the latent period for the response of the pancreas varied in these experiments from 5 to 10 minutes.

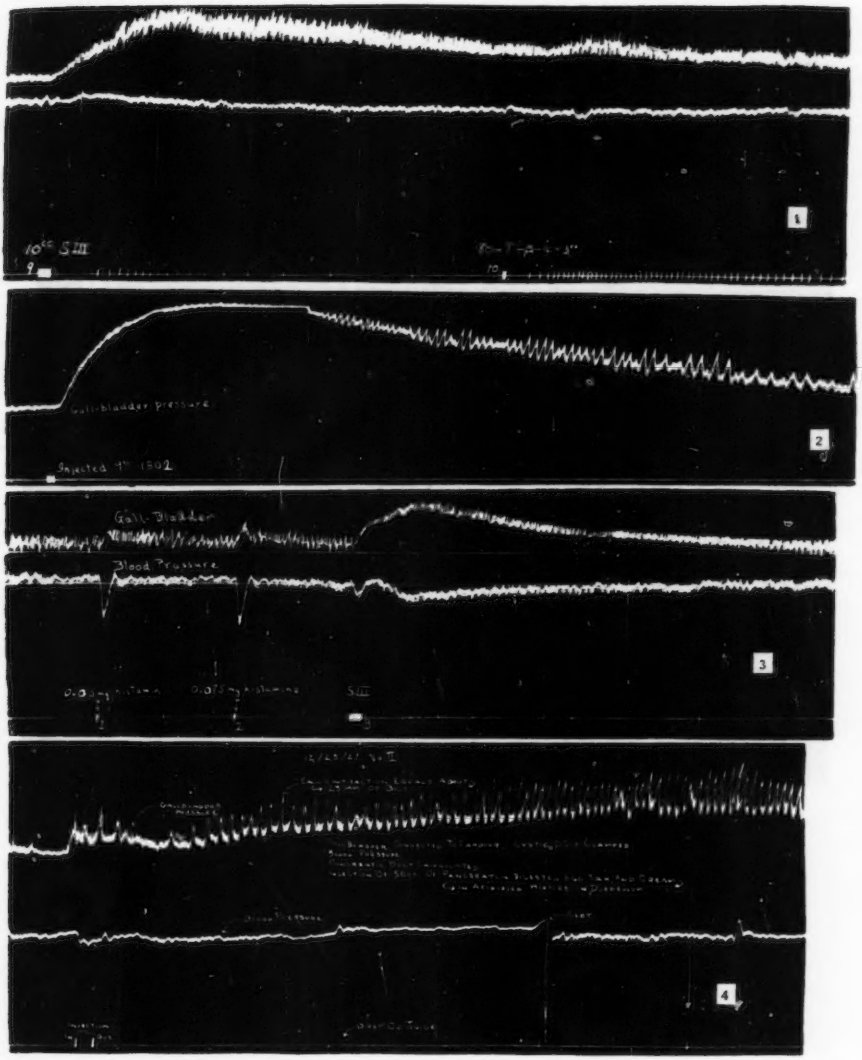
On contraction of the gall bladder caused by other agents introduced into the duodenum. In no experiment were we able to cause a marked and sustained change in the gall bladder by injecting olive oil into the duodenum. The same was true of cream and egg-yolk. The injection of butter was attended with more success. But if the cream, olive oil and egg-yolk were digested with "pancreatin" previous to injection, contraction occurred.

Fig. 1. Dog under barbital anesthesia with the cystic duct clamped, fundus of the gall bladder cannulated. The upper record is a tambour tracing of the intra-gall-bladder pressure. The record just below it is the blood pressure. The lower line shows the base line of the blood pressure, the point and time of injection and drops of pancreatic juice. At *9* 10 cc. of preparation S III were injected intravenously. At *10* 3 cc. of preparation 80-t-p-i were injected. The latter preparation contained 1 mgm. of solid material per cubic centimeter, and comparably more secretin and less cholecystokinin than preparation S III.

Fig. 2. Dog under barbital anesthesia with cystic duct clamped and the fundus of the gall bladder cannulated. Nine cubic centimeters of preparation 1802 were injected intravenously. This amounted to four threshold doses. It contained no vaso-dilatin. The period shown on the tracing was approximately twenty minutes. The bile rose in the manometer 11.5 cm. This tracing was the result of the demonstration before the North Chicago Branch of the Chicago Medical Society.

Fig. 3. Dog under barbital anesthesia with cystic duct clamped, the fundus of the gall bladder cannulated, and the pancreatic duct cannulated. This tracing contrasts the effect of histamine and a cholecystokinin preparation.

Fig. 4. Dog under barbital anesthesia with the cystic duct clamped, the fundus of the gall bladder cannulated and the pancreatic duct cannulated. The tracing shows contraction of the gall bladder with rhythmic contractions (approximately 3 per minute) caused by the injection into the duodenum of 50 cc. of "pancreatin" digested egg-yolks and cream.



Figs. 1–4

One-half per cent butyric acid and five per cent soap solution when injected into the duodenum caused a contraction.

PROOF OF A HORMONE. *On the results of cross-circulation experiments.* Four cross-circulation experiments (carotid-to-carotid) have been performed, three yielding positive results and one negative.

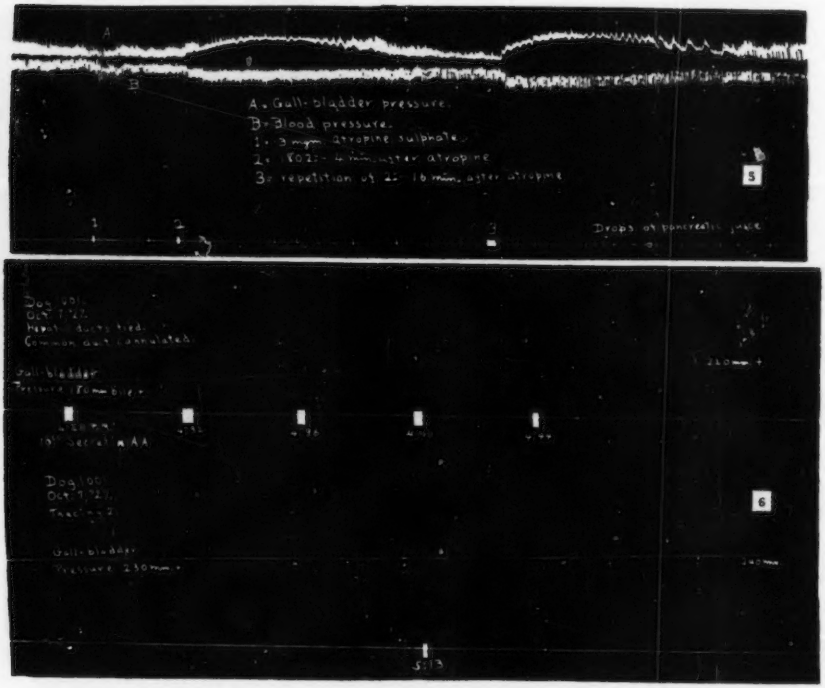


Fig. 5. Dog under barbital anesthesia, cystic duct clamped, fundus of gall bladder cannulated, and the pancreatic duct cannulated. The tracing shows that atropine does not prevent the action of cholecystokinin. Atropine may possibly slightly decrease its action, but we have not performed a sufficient number of experiments to definitely settle this question.

Fig. 6. Twenty-four hours previously the hepatic ducts were tied and the common bile duct was cannulated. The cannula was connected to a manometer and a recording tambour. Injections were made at the times indicated. Note the steplike rise in pressure from an original pressure of 180 mm. of bile to a pressure of 240 mm. of bile. The pressure began to decrease about 5:30.

Dogs of a like size were chosen whose bloods were shown to be compatible by agglutination tests. They were anesthetized with barbital and placed on the table side by side. The abdomen of each dog was opened, the

cystic duct clamped, the gall bladder and the pancreatic duct cannulated. The gall-bladder cannula was connected to a recording tambour. The lateral carotids were connected for a blood pressure tracing, and the two medial carotids were connected by cannulas for a cross-circulation. (See article by Ivy, Lim and McCarthy, 1925.) Simultaneous records of the blood pressure, intra-gall-bladder pressure, and pancreatic flow of both dogs were made.

In three out of four experiments the introduction of 40 cc. of N/10 HCl into the duodenum of the "first" dog caused the gall bladder of the "second" dog to contract. The gall bladder of the "second" dog, or the one that did not receive acid in the duodenum, contracted after a period of eight minutes in one animal, ten minutes in a second animal, and twelve minutes in the third animal (figs. 9 and 10).

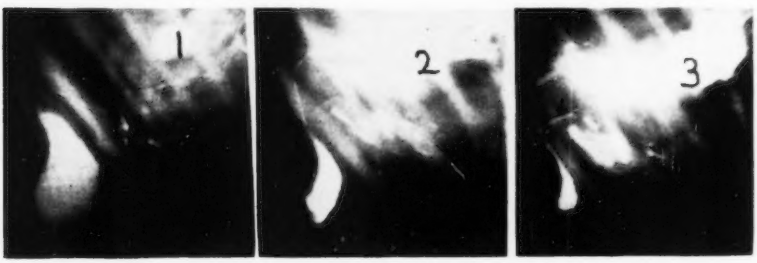


Fig. 7. Roentgenogram *1* was made before the injection of cholecystokinin; *2* was made one-half hour later after four injections at 10-minute intervals; *3* was made one hour after *1* after seven injections. Note the injection of the hepatic duct of the right lower lobe in *3*. Gall bladder filled with iodized oil 18 hours previous to the experiment.

These experiments demonstrate that the introduction of the acid into the duodenum of the "first" animal caused something to enter the blood, which passed via the cross-circulation cannula to the "second" animal, causing the gall bladder of the "second" animal to contract.

The pancreas of the "second" dog was only stimulated in one of the four experiments.

Discussion. Our results show that by the use of appropriate methods, a "purified" extract of the intestinal mucosa can be made containing a substance which on intravenous administration causes the gall bladder to contract and evacuate. That the substance is not histamine is shown by the fact that its injection causes no fall of the blood pressure. That the substance is not choline is shown by the fact that its injection is not antagonized by atropine in addition to the fact that blood pressure does not fall.

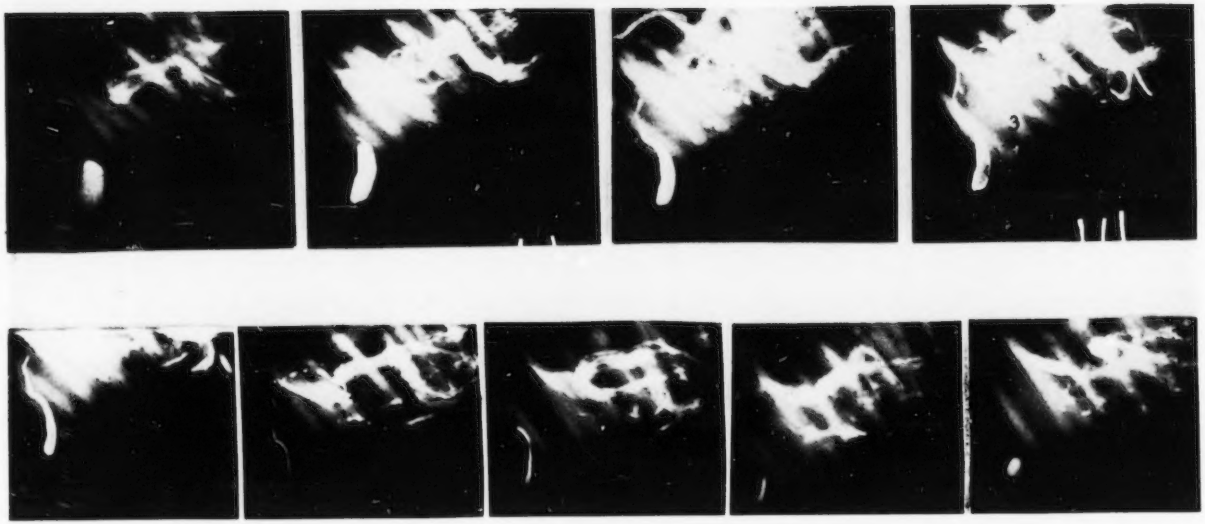


Fig. 8. *1*, control at 9:20; *2*, 10 minutes after first injection. Note iodized oil in duodenum; *2a*, 9:33, 2 minutes after 2nd injection; *3*, 9:40, 2 minutes after 3rd injection (note slight injection of right hepatic duct); *4*, 9:50, 2 minutes after 4th injection (can follow duct to duodenum); *5*, 10:00, 2 minutes after 5th injection; *6*, 10:10, 2 minutes after 6th injection; *7*, 10:20, 2 minutes after 7th injection. Injections were then stopped. One-half hour later *8* was taken. Note the change in contour denoting that the gall bladder has probably relaxed. The dog was immediately etherized. The gall bladder contained about 2 or 3 cc. of iodized oil in a viscid mass, and 4 or 5 cc. of apparently "fresh" bile.

The chemical properties of this substance, as far as they are known, will be given in a later paper.

After some consideration, we have decided to name the substance in

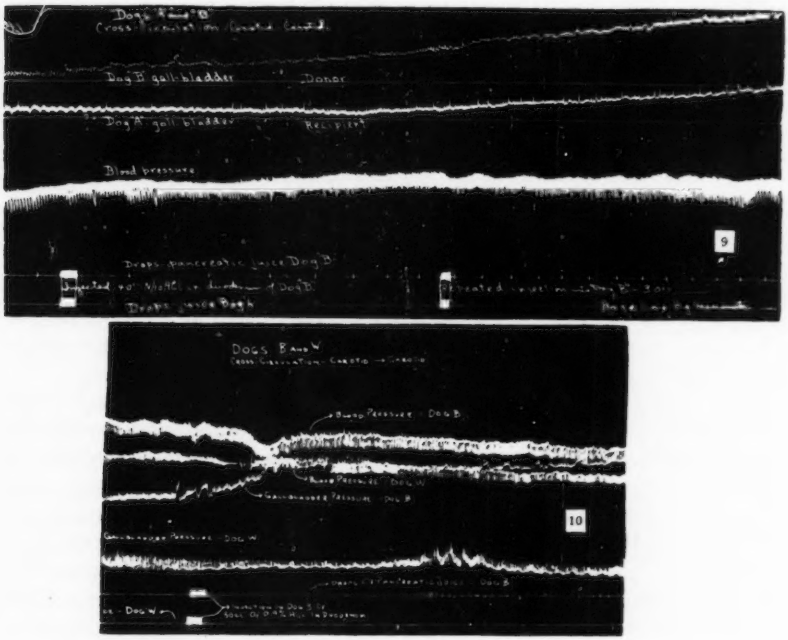


Fig. 9. Tracing from cross-circulation experiment 3, showing that about ten minutes after the introduction of acid (N/10 HCL, 40 cc.) in the duodenum of dog B (donor) the gall bladder of dog A contracted. A second injection of acid into the duodenum of dog B was made in order to obtain a maximal production of hormone. Note that the acid caused the gall bladder of dog B to contract very soon after the injection was made. Note that the blood pressure of these two animals was practically identical, one record being superimposed on the other. A very slight "crossed" pancreatic response occurred in this experiment.

Fig. 10. Tracing from cross-circulation experiment 2, showing a slight "crossed" effect which occurred about eight minutes after the acid had been injected into the duodenum of dog B. Note the marked contraction of the gall bladder in dog B which occurred very soon after the acid was injected. Figure 9 shows the strongest and figure 10 the weakest "crossed" effect in the three positive results of our four cross-circulation experiments.

intestinal extracts which causes the gall bladder to contract, "cholecystokinin" (that which excites or moves the gall bladder.) Some evidence will

be presented in a later paper which leads us to believe that the gall bladder contracting principle is different from "secretin".

The maximum power for the gall bladder to contract caused by injection of "cholecystokinin" under our experimental methods is 24.0 cm. of bile pressure. This figure agrees closely with the figures reported by Higgins and Mann (1926). Three hours after a meal, they observed a maximum pressure of 26.0 cm. of bile; the average pressure maintained for 30 or 40 minutes was 21.0 cm. Our average figure is approximately 10.0 cm. of bile on the anesthetized dogs, and in the three unanesthetized dogs, approximately 20.0 cm. (Mann, 1924).

We have observed rhythmic contractions of the gall bladder to occur spontaneously. They almost always occur after an injection of "cholecystokinin," if they have not been present before, and if present before are, as a rule, increased by the injection. Frequently, a small dose of "cholecystokinin" may not cause a tonic contraction, but will initiate or increase the amplitude of the rhythmic contractions. They occur at a rate of from 2 to 4 per minute (Mann, 1924). We have observed rhythmic contractions which have amounted to as much as 3.0 cm. of bile pressure.

We have interpreted the injection of, or the backing up of the lipiodol into the hepatic ducts, as being due to the pressure of the duodenum on the intramural portion of the common bile duct, or to a hypertonic sphincter of Oddi. The literature (Mann, 1924) shows that a minimum pressure of 5.0 cm. and a maximum of 67.5 cm. of water is necessary to overcome the resistance of the sphincter of Oddi, or the intramural portion of the common bile duct. It is obvious, then, whether or not the gall bladder contraction leads to evacuation of the gall bladder depends on the amount of resistance of the sphincter or intramural portion of the common duct. If the duodenum were spastic, it is quite likely that the gall bladder would not evacuate even though caused to contract by "cholecystokinin" (see Boyden, 1928). This, we believe, is the most probable explanation of the negative results obtained by Whitaker (1927) on the introduction of acid into the duodenum of man.

We have never observed the gall bladder of the dog to be completely empty, and we have not been able to empty it completely by injections of "cholecystokinin." Because of the anatomical attachments and relations of the gall bladder it is difficult to conceive of complete emptying except by some mechanism causing very gradual suction.

The results of cross-circulation certainly demonstrate a hormone action. We know of no other way of accounting for our results other than that the acid in the duodenum caused something to enter the blood. We used relatively large quantities of acid in order to get a maximum formation of the hormone. A maximum formation of the hormone is necessary for

such an experiment because of the dilution in the "recipient's" blood of only the hormone that flows through one carotid of the "donor." Under ideal conditions, which we believe we had in our experiments, there would be a minimum dilution of at least one to twenty. Even then, the hormone flows through an additional capillary bed (the head). We believe the positive results observed are unequivocal.

The observed fact that there was an apparent relation between the tension of the gall bladder, or the pressure exerted on its wall, and the amount of contraction elicited is not contrary to the physiological properties of smooth muscle. The best explanation that we can offer for the observation that the gall bladder of the "fed" dog did not respond as well as that of the "starved" dog (24 hours), is that the gall-bladder musculature had been either almost maximally excited or fatigued by the hormone produced by the meal. The amount of bile present in the gall bladder of these "fed" dogs was less than that we observed to be in most of the "starved" dogs. The "pressure head" against which the gall bladder contracted, from 5 to 6 cm. of bile, was the same in both "starved" and "fed" dogs. We have not performed experiments on the effect of varying the pressure in the gall bladder of the "starved" dogs, or with the attempt to analyse this phenomenon.

The fact that we observed no contraction of the gall bladder on the introduction of olive oil, cream and egg-yolks into the duodenum, and that a slight contraction occurred on the introduction of butter, 0.5 per cent butyric acid, 5 per cent soap solution, and that a marked contraction occurred on the introduction of digested (pancreatin) egg-yolk and cream, argues that before fats, egg-yolk, etc., stimulate the formation of a hormone or cause gall bladder contraction they must be digested. This is not necessarily true, however, because our dogs were anesthetized and in such a condition only strong or maximal stimuli may cause action. According to Boyden (1926b, 1928) the gall bladder begins to empty within fifteen minutes after ingestion of a meal. Elman and McMaster (1926) and Boyden (1928) report that the smelling or sight of fried bacon causes a change in the gall bladder and some ejection of bile, which Whitaker (1927) was unable to confirm. These observations are mentioned to call attention to the very likely possibility that other mechanisms than a hormone mechanism are concerned in causing gall-bladder evacuation.

SUMMARY AND CONCLUSIONS

1. An extract of the upper intestinal mucosa has been shown on intravenous injection to cause contraction and evacuation of the gall bladder. The extract (prepared by Ivy, Kloster and Lueth) is free of vaso-dilatin and has no objective toxic effect on unanesthetized dogs.

2. Cross-circulation experiments show that when acid is injected into the duodenum, something gets into the blood which causes the gall bladder to contract. We believe this observation proves a hormone mechanism for gall bladder contraction and evacuation. It is pointed out that other mechanisms may be concerned in gall bladder evacuation.

3. We propose the term "cholecystokinin" as a name for the hormone and the active substance in intestinal extracts, which causes the gall bladder to contract and evacuate.

4. The amount of pressure that results from the contraction of the gall bladder under the influence of "cholecystokinin" is for one injection a maximum of 11.5 cm. of bile pressure and a minimum of 1.0 cm., and for a series of injections an average of 10.0 cm. and a maximum of 22.5 cm. in anesthetized dogs; and in unanesthetized dogs for a series of injections it is an average of 20.0 cm. of bile and a maximum of 24.0 cm. of bile. The average duration of the contraction for a single dose is 15 minutes. Following a series of doses the gall bladder relaxes in from 30 to 60 minutes.

5. The injection of the following substances into the duodenum of the dog caused the gall bladder to contract with the animal under light barbital-ether anesthesia: 15 to 40 cc. of N/10 HCl, 30 cc. of butter, digested egg-yolk, cream and olive oil, 0.5 per cent butyric acid, and 5 per cent soap solution. Undigested olive oil, egg-yolk and cream were ineffectual.

6. Spontaneous rhythmic contractions of the gall bladder were observed to occur from 2 to 4 times a minute. A small dose of "cholecystokinin" would usually increase their amplitude to as much as 3 cm. of bile pressure; a large dose would usually cause them to disappear at the height of the contraction, but they would reappear some time during the period of relaxation. If they were not present prior to the injection, they would frequently appear during the latter part of the period of relaxation.

7. We have observed the hepatic ducts to be injected with lipiodol during the contraction of the gall bladder, the injection of the ducts being due, we believe, to increased (abnormal?) tone of the duodenum or sphincter of Oddi.

We desire to acknowledge the assistance of Dr. Grant Kloster and Mr. H. C. Lueth in performing a number of our experiments.

Preliminary reports: Proc. Soc. Exp. Biol. Med., xxv, 113, 1927; Ibid., xxv, 251, 1928; Journ. Amer. Med. Assoc., lxxxx, 445, 1928.

Demonstrations: Physiology Seminar, University of Chicago, November 23, 1928; North Shore Branch of Chicago Medical Society, April 5, 1928.

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STUDIES ON THE KNEE JERK

VII. The Effect of Moderate Doses of Ephedrin Sulphate on the Knee Jerk

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Early work upon the pharmacological action of ephedrin (1) showed this drug to be a central nervous system stimulant, for among other things the injection of the drug produced convulsions.

Chen (2) noted in 1924 that small doses (40 to 60 mgm.), when used clinically, caused a slight throbbing in the head. In a more recent paper (3) he reports that, "Some patients, especially women, after receiving 200 mgm. of ephedrin by mouth, complain of some of the following symptoms: perspiration, chill, palpitation, weakness, dizziness, nervousness, tremor, nausea and epigastric discomfort." He further states that, "The most probable explanation of these effects is stimulation of the central nervous system."

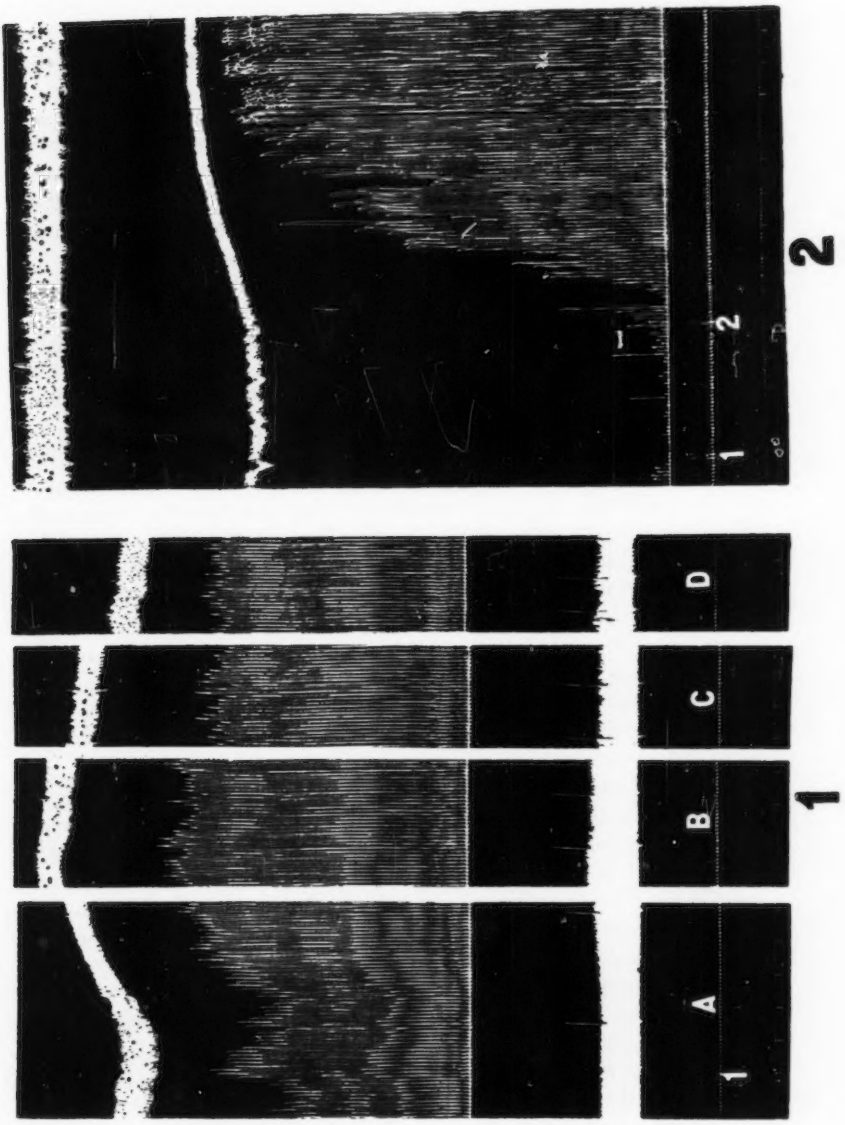
Miller (4), Rowntree and Brown (5), Piness and Miller (6) and others noted similar manifestations of central nervous system stimulation when using the drug therapeutically in the treatment of various diseases. Howard and Lee (7), using the drug as a mydriatic in concentrations over 5 per cent, observed in several patients that mild symptoms of distress occurred following instillation into the conjunctival sac.

Nagel (8), found that a few rabbits tolerated a dose of 40 mgm. of ephedrin per kilo intravenously showing only an increased excitability.

[1] Aided by a grant from the Billings Medical Club Research Fund, Chicago, Illinois.

Fig. 1. Showing the effect of injecting subcutaneously 50 mgm. of ephedrin HCl on the blood pressure, knee jerk and respiration of a dog weighing 8 kgm. and anesthetized with 2.5 grams barbital-Na. Cord intact. A = at *1*, injection of the ephedrin. Note the improvement of knee jerk and elevation of blood pressure at the end of 5 minutes. B, C and D, ten, twenty and forty minutes respectively after A.

Fig. 2. Showing the effect of injecting subcutaneously 100 mgm. of ephedrin HCl (in a dog under barbital-Na anesthesia) on the respiration, blood pressure and knee jerk. Cord transected at the 12th thoracic segment. *1*, subcutaneous injection of 100 mgm. of ephedrin-HCl. *2*, massage of the injected area.


2
1
2
1
A
B
C
D
1

One guinea pig (720 grams) was given 25 mgm. of ephedrin intravenously. It became very restless and had dyspnea. After a time it ejected sperm but after two minutes became quiet and showed only an increased respiratory rate.

In the *Preliminary Report of the Council on Pharmacy and Chemistry on Ephedrin* (9) one physician reported that three patients out of twenty experienced nausea, bladder irritation and headache from the administration of 200 mgm. of ephedrin per day. In a subsequent report (10) it was noted that the oral administration of ephedrin generally causes symptoms similar to an anxiety complex.

Reports of the excitatory nature of ephedrin reached us from local physicians. Having a suitable apparatus for quantitatively determining cord stimulation, it was thought advisable to study the action of ephedrin on the excitability of the spinal cord.

METHODS. Knee jerks were taken by means of a special knee jerk apparatus described in a previous paper by one of us (11). It will be recalled that this device automatically delivers a constant blow to the patellar tendon at a definite interval (7 seconds) and records the response upon a smoked drum. Also, it is possible to take records over a long period of time, thereby obtaining a suitable control period of a great number of responses as well as recording a great many responses during and following the experimental procedures.

Dogs (12–20K) under barbital-Na anesthesia (0.25 gram per kilo intraperitoneally) with cords intact, or with acute section of the cord in the twelfth thoracic region were used in these experiments. The animals were arranged for taking knee jerks as described in a previous paper of this series (11). Simultaneous records were made of knee jerks, carotid blood pressure and respiration.

A typical experiment consisted of: 1, a suitable control period using a submaximal blow to the patellar tendon followed by 2, either an intravenous or subcutaneous injection of ephedrin HCl (12½ to 100 mgm. per dog), and 3, a period of several hours showing the result of injection of the drug. In some of the experiments small doses of strychnine sulphate (½ mgm. per dog) were injected merely to confirm our conclusions that the ephedrin had a strychnine like action of the cord.

RESULTS. Figure 1 shows a typical tracing obtained from a dog with the cord intact. Following the injection of 50 mgm. of ephedrin subcutaneously there was an improvement of the knee jerk which lasted for several hours. With the return of the blood pressure to the normal level there was not a return of the knee jerk to the lower amplitude obtained during the control period showing that the improved circulation was not primarily the cause of the higher kick, since the increased excitability of the cord, as judged by the height of the knee jerk, remained long after the blood pressure had returned to the normal level.

Figure 2 shows a record obtained from a dog with an acute transection of the cord in the region of the twelfth thoracic vertebra. In this experiment there was a very marked improvement of the knee jerk following the subcutaneous injection of 100 mgm. of ephedrin with but a slight elevation of the blood pressure. This improvement persisted throughout the course of the experiment which lasted for several hours, even after the blood pressure had returned to normal level again showing that the increased knee jerk was not the direct result of an improved circulation.

In these animals small doses of strychnine ($\frac{1}{2}$ to 1 mgm. per dog), would also cause an increase of the knee jerk, but the effect was more transitory in nature.

In several experiments ephedrin was effective when strychnine was not, suggesting that the point of action on the cord of these two drugs is possibly different.

Following the injection of ephedrin there seemed to be a tendency for the animals to recover partially from the depression caused by the barbital-Na; for the lid reflex became very brisk, and sometimes spontaneous movements of the head and limbs occurred. The animals were aroused from their sleep, as previously noted by Airila (12) and Morita (13).

SUMMARY AND CONCLUSION

These experiments show that ephedrin HCl, in moderate doses ($12\frac{1}{2}$–100 mgm. per dog) causes a marked increase in the reflex excitability of the spinal cord, (as measured by the knee jerk); and that this action is not dependent on any influence of the higher centers on the cord, nor due to the hemodynamic action of the drug.

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CHANGES IN BLOOD SUGAR FOLLOWING INJECTIONS OF PEPTONE IN THE DOG

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Reports in the available literature on the effect of injections of peptone on blood sugar show a striking lack of uniformity. Henderson and Underhill (1) reported glycosuria probably associated with hyperglycemia in dogs following the injection of peptone, McGuigan and Ross (2) obtained a hypoglycemia in peptone shock in dogs. They suggested that the hyperglycemia reported by other workers was due to the ether anesthesia, because when the administration of ether was stopped during the course of the experiment they obtained a fall in blood sugar, which rose again with readministration of ether. However, when ether was given throughout the entire experiment there was either a fall, or no change in blood sugar. Kuriyama (3) found an increase in blood sugar of a few hours' duration in rabbits injected intravenously with peptone. Zeckwer and Goodwell (4) reported a hyperglycemia in rabbits in bacterial anaphylaxis. Maud L. Menten and Helen M. Manning (5) concluded that different samples of Witte's peptone, when injected intravenously in rabbits, vary widely in the manner in which they modify glycemia, the changes in the blood sugar ranging from varying degrees of hyperglycemia or lack of appreciable change to fatal hypoglycemia. Alterations in the circulation of the blood occur in peptone shock in both dog and rabbit. But the resultant changes in blood distribution are different in the two species. These changes in distribution may be an underlying factor in differences in blood sugar in these animals.

In the experiments here reported dogs were anesthetized with ether and kept under a reasonably constant moderately deep anesthesia throughout the entire experiment. The femoral veins were exposed, a cannula connected with a manometer was ligated in the carotid artery, and an incision about 4 inches long was made in the median line of the abdomen. Through the latter, changes in the size, consistency and color of the liver could be observed. Samples of blood were taken from the hepatic and femoral (or jugular) veins before, and at frequent intervals after, the intravenous

injection of Witte's peptone. Blood sugar determinations were made according to the Folin-Wu method (8). Hyperglycemia caused by ether has been reported by Epstein and Aschner (6), Epstein, Reiss and Branower (7), Mann and others. However, ether was the most convenient and efficient anesthetic for our investigations, and since it causes a slow and gradual rise in blood sugar the changes brought about by the injection peptone were quite clear.

Some investigators have considered dogs unsuited for work on blood sugar because of the marked variations in sugar content of the blood of supposedly

TABLE 1

Showing the relation blood sugar and blood pressure in peptone shock in dog 2

TIME	BLOOD PRESSURE	BLOOD SUGAR, MGM. PER 100 CC.
	mm. Hg	
Before injection	120	140
1 minute after injection	78	135
2 minutes after injection	40	130
3 minutes after injection	44	127
5 minutes after injection	56	150
8 minutes after injection	72	190
14 minutes after injection	82	212
19 minutes after injection	94	212

TABLE 2

Showing the maximum and minimum changes in blood sugar in peptone shock

	BLOOD SUGAR BEFORE PEPTONE	BLOOD SUGAR AFTER PEPTONE	
		Minimum	Maximum
Dog 1	126	111	187
Dog 2	140	127	212
Dog 3	114	86	274
Dog 4	160	129	189
Dog 5	142	132	277
Dog 6	86	65	92

normal animals. Bang's (9) figures range from 40 to 220 mgm., and Macleod's (10) from 73 to 219 mgm. of sugar per 100 cc. of dog's blood. Our figures, 79 to 200 mgm. per 100 cc., correspond rather closely to Macleod's. These variations are probably due, as pointed out by Shaffer (11), to the fear and excitement incident to anesthetizing the dog. The above facts materially reduce the value of experiments on blood sugar in anesthetized dogs when comparison is made between results in different animals. In our work, however, study was made of the alterations in the level of blood

sugar in the same anesthetized animal kept under approximately constant experimental conditions. The results are, therefore, comparable. It is permissible also to compare results in different animals in our series at similar stages of the experiments because these averages indicate *relative changes* in values.

In general, we found that during peptone shock in the dog there is either very little change or a moderate fall in blood sugar below the level immediately preceding the shock. At times a slight increase was observed in the

TABLE 3

Blood sugar values in hepatic and femoral veins in dog 3 in peptone shock

	BLOOD SUGAR	
	Hepatic vein	Femoral vein
Before injection	163	160
2 minutes after injection		148
3 minutes after injection	288	129
8 minutes after injection		131
Liver relaxing		
18 minutes after injection	294	190

TABLE 4

Showing the maximum and minimum changes in blood sugar in hepatic and femoral veins in peptone shock

	BLOOD SUGAR BEFORE INJECTING PEPTONE		BLOOD SUGAR AFTER INJECTING PEPTONE			
			Hepatic vein		Femoral vein	
	Hepatic vein	Femoral vein	Minimum	Maximum	Minimum	Maximum
Dog 3	163	160	288	294	129	190
Dog 4	142	144	184	277	132	284
Dog 5	96	97	126	198	88	113
Dog 6	131	112	125	180	106	136
Dog 7		114	250	302	86	274

first sample of blood drawn within a minute after the injection of the peptone. This was not constant, however. The curve of blood sugar values roughly paralleled that of the blood pressure, that is, they were lowest when the blood pressure was lowest. As the blood pressure rose and the animal gradually recovered from the shock the blood sugar values also increased. The quantity of sugar in the blood paralleled very closely the state of engorgement of the liver. The most marked reduction in blood sugar coincided with the maximum engorgement of the liver. Increase in blood sugar

followed the subsidence of the hyperemia of the liver and reached its maximum after the liver returned to its normal size. Upon complete recovery from the shock, and while still under anesthesia, all of the animals showed a rise in blood sugar considerably above the initial value. These changes in blood sugar have certain striking similarities to those induced by mechanical constriction of the hepatic veins, as described by us (12).

In the second series of experiments with a more potent sample of Witte's peptone we compared samples of blood taken from the hepatic veins with samples taken at the same time from the femoral or jugular veins. Blood pressure tracings were not always taken but changes in the liver were observed. Blood from the femoral veins showed changes similar to those described in the first series. Blood from the hepatic veins showed a marked increase in sugar content at the time the liver was most distended and its capsule most tense. In other words, at a time when blood coming from the liver showed a marked increase over the sugar content before the injection of peptone, that in the general circulation showed a definite decrease. Samples taken as the animal was recovering from the shock and the liver relaxing, showed an increase in sugar content of blood from the femoral vein and at the same time either a decrease or no change in that from the hepatic veins. In other words the two values approached each other (see tables 3 and 4).

All dogs of this series gave the same type of reactions except one, (dog 6), in which there was a less marked difference in the sugar content of bloods from the hepatic and femoral veins. Before the injection of peptone blood from the femoral and hepatic veins contained 86 and 85 mgm. of sugar per 100 cc. respectively. During shock blood from the femoral vein reached a minimum of 65 mgm. and that from the hepatic vein a maximum of 110 mgm. per 100 cc. In this animal it was definitely noted that there was but very slight increase in size of liver with very little congestion.

SUMMARY AND CONCLUSIONS

The above experiments indicate that in anesthetized dogs peptone causes a hypoglycemia in the peripheral venous blood during the period of low blood pressure when the liver is engorged and tense. When the blood pressure rises and the liver becomes less tense there occurs a rather marked rise in the blood sugar.

At the time in peptone shock in the dog when the liver has reached its greatest size and is most markedly engorged with blood there is a marked increase in the sugar content of the blood from the hepatic vein, while at the same time there is a decrease in that of blood from the femoral vein.

As long as the circulation in the liver is free and unimpeded there is only a moderate difference in sugar values of blood from the hepatic and femoral

veins, an average in our series of 133 and 125 mgm. respectively. In peptone shock the obstruction to the free flow of blood through the liver with the attendant congestion and local stasis appears to cause an increased glycogenolysis in the liver. During the height of peptone shock so little blood leaves the liver (13) that, although the sugar content of such blood is quite high, the normal blood sugar level of the general circulation cannot be maintained and a hypoglycemia results. As the obstruction is relaxed during recovery the increased sugar in the liver enters the general circulation causing a hyperglycemia.

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CHANGES IN THE BLOOD RESULTING FROM MECHANICAL OBSTRUCTION OF THE HEPATIC VEINS

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In a former paper (1) we described a method of blocking the outflow of blood from the liver by mechanically constricting the hepatic veins. This method makes possible a study of some of the functions of the liver and has advantages over the more drastic methods formerly employed of removing the liver entirely or of permanently shutting it out of the circulation by producing an Eck fistula in one or more stages. The mechanical constriction of the hepatic veins can be done with less trauma than complete removal of the liver and is not, therefore, complicated by shock. It makes possible the temporary exclusion of the liver and splanchnic region from the general circulation and their return to the circulation at will, a thing that cannot be done in the case of Eck fistula.

In this paper we propose to present briefly some of the changes which occur in the blood as a result of temporary exclusion of the liver and splanchnic region from the general circulation and those which follow the release of the mechanical constriction which accomplished the exclusion. Some of these changes are being studied further. They are here presented as observed facts.

1. EFFECT ON BLOOD PRESSURE. The changes in blood pressure induced by this procedure have been described in previous papers (1), (2). They consist in a precipitate fall in arterial pressure of from 40 to 60 mm. Hg. The pressure quickly reaches a level which remains reasonably constant for 20 minutes or more. Upon release of the constriction the pressure rises immediately to a level usually about 10 mm. higher than that before constriction and then returns to normal in a half-minute or less.

2. EFFECT ON CONCENTRATION OF THE BLOOD. The method used in estimating the changes in the concentration of the blood in the general circulation was that described by Lamson and Roca (3) and others and previously employed by one of us (4). While not wholly free from criticism it yields comparable results. Two cubic centimeters of blood were drawn in a clean dry syringe from the femoral or jugular veins and prevented from coagulating by the addition of dry sodium citrate. The tech-

nique consisted in measuring 0.25 cc. of each sample of blood into 25 cc. of 0.25 per cent ammonia solution. The red cells were promptly laked and the solution was quite clear. Carbon monoxide was bubbled through these solutions until all of the hemoglobin was changed to carbon monoxide hemoglobin. The sample of blood taken before constriction of the hepatic veins was taken as the normal control and its value placed arbitrarily at 100. The subsequent samples were compared with this normal in a colorimeter and their values stated in percentages of the normal.

In addition to the control samples of blood others were taken at approximately 5 minute intervals after 1, constriction; 2, release; 3, reconstriction, and 4, a second release. In most animals a rapid decrease in blood concentration occurred in the first 5 minutes after constriction and was followed by a gradual partial recovery. After release of the constriction the concentration usually showed an increase; in one animal it rose well above the original. A characteristic result is shown in the following table (dog H. V. 7).

	Blood Concentration *per cent*
Before constriction	100
5 minutes after constriction	83
10 minutes after constriction	75 (?)
15 minutes after constriction	96
5 minutes after release	92
15 minutes after release	98
5 minutes after reconstriction	86
10 minutes after reconstriction	85
5 minutes after release	97

The averages of results in six dogs at corresponding intervals were as follows:

	Blood Concentration *per cent*
Before constriction	100
5 minutes after constriction	89
10 minutes after constriction	95
15 minutes after constriction	98
5 minutes after release	99.9
10 minutes after release	97
15 minutes after release	97
5 minutes after reconstriction	89
10 minutes after reconstriction	93
5 minutes after release	98

The above findings indicate that the blood becomes more dilute very quickly after complete mechanical obstruction of the hepatic veins and then gradually increases in concentration. One of us (5) has called atten-

tion to the marked increase in outflow of lymph from the thoracic duct during mechanical constriction of the hepatic veins, an average increase of 5.2 times the normal. Fluid also probably enters the blood from the tissues as in low blood pressure from hemorrhage. Fluid from these two sources may account for the dilution of the blood during the first 5 or 10 minutes after constriction of the hepatic veins. The reason for the gradual increase in concentration during constriction is not so clear. Three possible sources of red cells may be concerned. 1. After the first few minutes of mechanical constriction of the hepatic veins the fluid from the thoracic duct contains some blood. 2. A general vasoconstriction may force more cells into the blood stream. 3. The increased pressure in the liver may force the constriction and deliver into the circulation a small amount of blood highly concentrated by loss of fluid into the lymph escaping from the liver through the thoracic duct.

3. CHANGES IN COAGULATION TIME. The coagulation time in these animals before constriction of the hepatic veins ranged from $2\frac{1}{4}$ to $4\frac{1}{2}$ minutes. Two methods were used in determining the coagulation time.

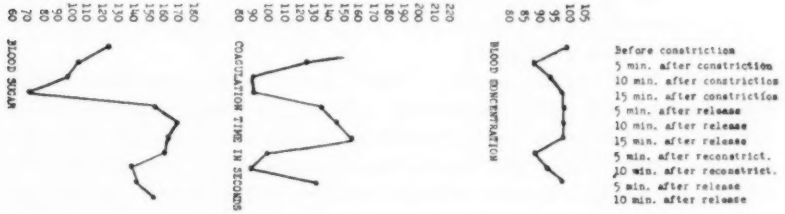

Fig. 1. Showing the changes in the blood during mechanical constriction of the hepatic veins.

Blood concentration is expressed in percentage of the concentration immediately preceding the mechanical constriction.

Coagulation time is expressed in seconds.

Blood sugar is expressed in milligrams of sugar per 100 cc. of blood.

1. That of Sabrazes: capillary pipettes were filled with blood and half-inch lengths broken off at intervals of 15 to 30 seconds. 2. That of Peterson (6) in which drops of blood held in loops of platinum wire are submerged in warm water. These two methods yielded closely comparable results. The figures given in the tables are averages of results by the two methods. Constriction of the hepatic veins caused a very distinct shortening of the coagulation time. A characteristic result is shown in the following table (dog H. V. 4).

	seconds
Before constriction	210
5 minutes after constriction	90
10 minutes after constriction	100

	seconds
15 minutes after constriction	100
5 minutes after release	225
10 minutes after release	205
15 minutes after release	210
5 minutes after reconstriction	95
10 minutes after reconstriction	100
5 minutes after release	195

The average results in 6 dogs were as follows:

	seconds
Before constriction	206
5 minutes after constriction	127
10 minutes after constriction	93
15 minutes after constriction	93
5 minutes after release	139
10 minutes after release	149
15 minutes after release	159
5 minutes after reconstriction	103
10 minutes after reconstriction	91
5 minutes after release	135

No explanation is offered for this phenomenon. Further investigations as to its cause are in progress.

4. CHANGES IN BLOOD SUGAR. Mann (7) and others have shown that total removal of the liver is followed by a fall in blood sugar to such a low level that hypoglycemic convulsions occur. Shutting the liver and splanchnic area out of the circulation by mechanically constricting the hepatic veins results in a surprisingly rapid fall in blood sugar. A characteristic result is shown in the succeeding table (dog H. V. 5).

	mgm.
Before constriction	96
5 minutes after constriction	65
10 minutes after constriction	65
15 minutes after constriction	50
5 minutes after release	100
10 minutes after release	103
15 minutes after release	100
5 minutes after reconstriction	97
10 minutes after reconstriction	96
5 minutes after release	117
10 minutes after release	110

The average for six dogs was as follows:

	mgm.
Before constriction	128
5 minutes after constriction	109
10 minutes after constriction	101

	mgm.
15 minutes after constriction	74
5 minutes after release	157
10 minutes after release	173
15 minutes after release	168
5 minutes after reconstriction	167
10 minutes after reconstriction	143
5 minutes after release	147
10 minutes after release	157

As soon as the constriction of the hepatic veins is released the blood sugar promptly rises to a level considerably higher than that before the veins were mechanically constricted. In one animal it rose to 435 mgm. per 100 cc., the original level having been 163 mgm. There was a slightly greater fall in blood sugar in the first ten minutes following the first constriction than in the similar interval after reconstriction,—an average of 28 per cent in the first instance against 15 per cent in the second. The average maximum fall in 15 minutes after constriction amounted to 42 per cent. Although it is not evident in the average results from 6 dogs, the blood sugar not infrequently reached a higher level after the second constriction than after the first. This is shown the case of dog H. V. 5 above.

SUMMARY

The following changes in the blood, shown graphically in figure 1, have been noted to follow mechanical constriction of the hepatic vein:

1. A precipitate fall in blood pressure of from 40 to 60 mm. Hg, a level which is then maintained reasonably constant during constriction for 20 minutes or more.

2. A noteworthy decrease in blood concentration (an average of 11 per cent in 6 dogs) with a gradual increase in concentration until, at the end of 15 minutes, approximately the normal value is reached.

3. A marked decrease in coagulation time during constriction followed by an increase upon release.

4. A rapid fall in blood sugar (an average of 42 per cent in 6 dogs) during constriction for 15 minutes with a rapid rise upon release.

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NEURO-MUSCULAR RESPONSE OF RATS TO GALVANIC STIMULI[1]

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Three quarters of a century ago Dubois-Reymond and Pflüger made fundamental contributions to electro-physiology. In subsequent years many applications were made to therapeutic and diagnostic procedures. Erb in 1878 described the use of the galvanic current as a measurement of neuro-muscular irritability in tetany. An account of the early work is given in Biedermann (1), and summarized by Howell (2). The application of these phenomena to the diagnosis of tetany has been studied by Holmes (3), and is widely used in pediatric clinics, in conjunction with the analysis of the blood serum for calcium and phosphorus.

There are four possible threshold values of galvanic current necessary to cause muscle contraction, the closing and opening of the cathode, and the closing and opening of the anode. These will be referred to in this communication by the abbreviations—C. C., A. C., A. O., C. O., which also gives the ascending order of the current normally observed. Occasionally, cathodal closing tetanus, C. C. T., is found.

PLAN. The white rat, which has served so many useful purposes in the laboratory, seemed to us to be specially suitable to such measurements. Rats permit the solution of many nutritional problems, and the neuro-muscular irritability under varying conditions is of physiological importance. We have, therefore, studied the response to galvanic stimulation of rats varying in respect to age, sex, weight, and diet, and also of rats in anesthesia, both surgical and fatal. Approximately 2000 observations were made on 200 rats.

PROCEDURE. The amount of current required to cause muscular contractions in humans varies from 1 to 20 milliamperes. This amount is far in excess of that which will stimulate the contractions of rat muscle, which we have found requires one milliampere or less. We therefore modified the apparatus, supplied by the Wappler Electric Company, by inclusion of a galvanometer in series, so that reactions could be measured in units of 0.02 milliampere.

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The procedure followed is similar to that used in testing persons. An indifferent electrode is moistened with saline and placed on the abdomen or upon one hind foot. The stimulating electrode, modified to fit on the inner side of the rat's ankle, is applied over the posterior tibial nerve of the other foot. The rats in these experiments are neither tied to a board, nor given any drugs, but are held gently in the hands. One hand is held

TABLE 1

The galvanic stimuli necessary for muscular contraction in male rats

AGE	AVERAGE WEIGHT	C. C.	A. C.	A. O.	C. O. OR C. C. T.	AVERAGE
days	*grams*	*milliamperes*	*milliamperes*	*milliamperes*	*milliamperes*	*milliamperes*
18	28	0.32	0.52	1.54	1.62	1.00
30	73	0.30	0.62	1.50	1.58	1.00
38	90	0.22	0.56	0.96	1.52	0.82
45	118	0.24	0.52	0.88	0.36	0.50
55	160	0.24	0.58	0.52	0.72	0.52
65	201	0.22	0.44	0.62	0.82	0.52
67	208	0.28	0.46	1.16	0.96	0.72
71	307	0.30	0.54	0.98	1.06	0.76
141	360	0.18	0.76	0.94	0.84	0.68
244	456	0.24	0.40	0.84	0.82	0.58

TABLE 2

The galvanic stimuli necessary for muscular contraction in female rats

AGE	AVERAGE WEIGHT	C. C.	A. C.	A. O.	C. O. OR C. C. T.	AVERAGE
days	*grams*	*milliamperes*	*milliamperes*	*milliamperes*	*milliamperes*	*milliamperes*
18	27	0.34	0.58	1.68	1.74	1.06
30	56	0.28	0.52	1.44	1.28	0.88
38	92	0.22	0.50	0.76	1.12	0.68
45	118	0.26	0.48	0.60	0.72	0.52
55	148	0.16	0.44	0.56	0.76	0.48
65	160	0.22	0.34	0.66	0.80	0.50
67	165	0.28	0.42	0.72	0.66	0.52
71	214	0.24	0.46	0.80	0.74	0.56
141	235	0.18	0.38	0.72	0.80	0.52
244	298	0.08	0.24	0.76	0.70	0.44

about the thorax, and the other grasps the tail and left hind foot. Under these conditions the animal does not struggle or become excited. Gloves may be used, but in our laboratory the rats are so used to handling that even a male of 500 grams is held bare handed. It is best to have four operators, one to hold the rat, one to hold the indifferent electrode, one to operate the stimulating electrode and observe the contractions, and a fourth to operate the rheostat and observe the amount of current. The

operator of the stimulating electrode grasps the right hind paw gently between the thumb and fore-finger of the left hand. The foot is thus extended. The moistened stimulating electrode is pressed over the posterior tibial nerve and contractions of the muscle on closing and opening the key are felt and the movements of the claws are seen. The latter are used to measure the threshold stimulus. The fourth operator can check the accuracy of the threshold current as observed by the third by moving the rheostat up or down repeatedly to see whether the same reactions are observed with a uniform amount of current. If either the stimulating or indifferent electrodes are moved a variable resistance is intro-

TABLE 3

The galvanic stimuli necessary for muscular contraction of rats fed vitamin deficient diets

SEX	AGE	AVERAGE WEIGHT	C. C.	A. C.	A. O.	C. O.	AVERAGE
Vitamin A deficient diet. Rats with definite xerophthalmia							
	days	*grams*	*milli-amperes*	*milli-amperes*	*milli-amperes*	*milli-amperes*	*milli-amperes*
Male	103	156	0.16	0.26	0.80	0.68	0.48
Vitamin B deficient diet. Rats placed on diet at age of 28 days, and had ceased growing							
Male and female	48	68	0.24	0.38	0.76	0.70	0.52
Vitamin D deficient diet. Rats placed on diet at age of 28 days, and were definitely rachitic							
Male	49	74	0.18	0.30	0.58	0.62	0.42
Female	49	74	0.16	0.40	0.58	0.82	0.46

duced and the current must be adjusted before readings can be made. The smoothness and speed of obtaining results depend upon all four operators. With a trained group the values for the four reactions can be obtained in 3 to 5 minutes. Duplicate determinations give close checks.

Diets. Rats fed upon Sherman's normal diet "B" were tested (4). This consists of 33 per cent whole milk powder, 65.7 per cent ground whole wheat and 1.3 per cent sodium chloride. It has been shown adequate for nutrition and reproduction.

Data were also secured from rats fed upon diets lacking vitamins A, B and D respectively. The vitamin A deficient diet (5) consisted of 18 per cent extracted casein, 2 per cent agar, 3 per cent of McCollum's salt mixture 185, and 77 per cent dextrin. The diet was irradiated and 0.5 gram of yeast given each rat daily.

The diet lacking the vitamin B complex consisted of 18 per cent meat residue, 50 per cent raw corn starch, 23 per cent crisco, 5 per cent cod liver oil and 4 per cent of Osborne and Mendel's salt mixture IV (6).

The modified diet of Steenbock and Black (7) was used for the vitamin D deficient diet. It consisted of 76 per cent yellow corn, 20 per cent wheat gluten, 1 per cent sodium chloride and 3.0 per cent calcium carbonate, to which was added lard to the extent of 10 per cent.

RESULTS. *Age and sex.* In table 1 are given the data for male and in table 2 for female rats aged 18 to 244 days. The average weights are also given. It is clear that the younger rats from 18 to 38 days require higher currents for stimulation than do the older rats. We have added in the

TABLE 4

Galvanic reactions of rats before and after death

	C. C.	A. C.	A. O.	C. O.	AVERAGE
	milli-amperes	*milli-amperes*	*milli-amperes*	*milli-amperes*	*milli-amperes*
Killed by ether only					
Before death	0.24	0.32	0.68	0.58	0.46
After death	0.22	0.54	0.82	0.98	0.64
Killed by ether and bleeding					
Before death	0.20	0.38	0.70	0.72	0.50
After death	0.26	0.50	0.74	1.00	0.62
Killed by chloroform only					
Before death	0.22	0.32	0.98	0.84	0.58
After death	0.22	0.46	0.86	0.80	0.58
Killed by ether and bleeding					
Before death	0.26	0.38	0.70	0.52	0.46
5 minutes after	0.26	0.52	0.82	1.18	0.70
30 minutes after	0.50	0.62	1.92	2.56	1.40

last column the average values of all the reactions. Such a treatment of the data places more emphasis on the cathodal and anodal opening contractions than on the closing contractions. This we believe is a useful method in practice because the latter show greater differences, under abnormal conditions.

Vitamin deficiency. In table 3 are shown data of animals fed upon diets deficient in vitamin A, or vitamin B, or vitamin D. These rats show no striking differences from those on the normal diet. The difference in weight between normal rats and those fed deficient diets, and the similar electrical reactions of both, indicates that neuro-muscular irritability is a function of age or of the development of the nervous system and not of body weight.

Anesthesia and death. We have made observations on normal rats in anesthesia and have found that the amount of current required to cause contraction is roughly doubled. Inasmuch as the depth of the anesthesia was not controlled more accurate quantitative data need not be given. In addition, a number of animals were killed by anesthesia. The data are given in table 4. The reactions before and after death are quite similar. Fifteen minutes after death the animals are distinctly less irritable and in one hour after death irritability to the currents we used had practically ceased. Animals killed with or without bleeding gave practically the same results. This indicates that long after the circulation and respiration have ceased to function the state of neuro-muscular irritability is retained.

SUMMARY

The white rat is a suitable laboratory animal for quantitation of neuromuscular irritability to galvanic currents. Quantitative data have been presented showing the amount of current required to cause muscular contraction, in relation to age, sex, weight, diet, anesthesia and death. Deficiency of vitamins A, B and D, respectively, does not materially alter the response to galvanic stimuli. The current required to cause muscular contraction is less with older rats than with young animals. Neuromuscular irritability in normal animals is probably a function of age and not of growth. The current required for stimulation varies from 0.5 to 1.0 milliampere.

We wish to thank Mr. W. E. Anderson, and Drs. R. W. Jackson and Helen T. Parsons for kindly furnishing us with the animals fed upon diets deficient in vitamins A and B.

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RICKETS IN RATS[1]

VIII. Rickets and Tetany

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In a previous communication (1) it was reported that when rats made rachitic on a high calcium-low phosphorus diet, were continued on the same diet with the addition of sufficient phosphate to make the diet normal, not only were they cured of rickets, but other phenomena occurred. The blood serum calcium became low and the phosphorus high. Some of the rats showed carpopedal spasm, or convulsions: of which, part recovered and part died. Was this condition tetany?

Tetany of the infantile type is associated with low serum calcium values. In the tetany which follows the removal of the parathyroid glands low values for serum calcium are also found. Binger (2) designated the condition found in dogs after intravenous injection of alkaline phosphates as tetany, as did Salvesen, Hastings and McIntosh (3) that after phosphate feeding. In their studies also the serum calcium was low. However, hyperventilation tetany is present when the calcium is not lowered (4) and, on the other hand, low calcium in certain types of kidney disease is present without tetany (5). Further, other types of convulsions than tetany may be produced by faulty diets, e.g., the convulsive state associated with advanced stages of vitamin B deficiency. Therefore, in addition to the calcium and phosphorus determinations in the blood serum it seemed necessary to obtain the corroborative evidence supplied by the galvanic reactions of Erb. This method has proved of great value in the diagnosis of tetany in infants. Holmes (6) has supplied data showing the extent to which, in tetany, the reactions are lowered. That rats may be used for quantitation of electrical responses has been shown (7). It seemed therefore desirable to study the electrical reactions when rats were cured of rickets.

Plan of experiment. Rats made rachitic on a high calcium-low phosphorus diet were cured by the addition of phosphates to the diet, by cod liver oil and by irradiation, or by a combination of the last two. The

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

electrical reactions were daily observed. Two weeks later the blood was analyzed for calcium and phosphorus and roentgenograms were taken.

PROCEDURE. Rats were fed Sherman's normal diet B. When the young were born they were kept with the mother until 28 days, both having access to the above diet. Young rats weighing 45 to 60 grams were then fed Steenbock and Black's diet 2965 (7), consisting of 76 per cent yellow corn, 20 per cent gluten flour, 1 per cent sodium chloride and 3 per cent calcium carbonate. To 100 grams of this diet were added 10 grams of lard. One group received Zucker's rickets-producing diet (8), consisting of 85 per cent patent flour, 10 per cent egg albumin, 2 per cent sodium chloride, 2 per cent ferric citrate and 2.8 per cent calcium lactate. The rats were maintained upon these diets for 21 days and then various curative procedures were initiated. The rats were divided into groups of 4 or 5. Cod liver oil, equivalent to 2 per cent of the diet was substituted for an equal amount of lard; or the corn from which the ration was made was irradiated for 20 minutes at a distance of 2 feet with a mercury vapor quartz lamp; or a combination of both cod liver oil and irradiated food was fed; or phosphates were added in sufficient amount so that in the complete ration the phosphorus was equal by weight to the calcium. This was prepared so as to make the resulting food potentially alkaline, neutral or acid; and diets similar to the last were made containing double the amount of phosphate.

The responses of the muscles of the hind leg to galvanic stimuli were observed frequently. The technique of making the tests has been described (9).

RESULTS. The animals which received cod liver oil and irradiated food, as judged by the histological examination of the bones, and the rise from low serum phosphorus to normal showed the characteristic improvement associated with cure (1). Their general behavior was normal or lethargic. They gained in weight and were docile and quiet. The x-ray pictures showed healed rickets.

The rats cured by additions of phosphate to the diet were strikingly different. The serum phosphorus became extremely high and the calcium low. They were very excitable after the first day. At a slight noise in the laboratory they were startled and jumpy, while the rickets controls in the nearby cages were not disturbed. Some showed carpopedal spasm. The hind and fore paws were clenched and inverted. When picked up, the paws went into a rapid twitching movement or clonic convulsions. The rats sometimes walked on their hind feet, sometimes on their front feet only, sometimes lay on their side apparently unable to walk. Some died in this condition or were found dead. After being in convulsions for several hours some rats recovered. They then appeared normal except for the excessive response on being startled.

TABLE 1

The galvanic stimuli necessary for muscular contraction of rats cured of rickets by various procedures

DIET	Ca:P IN DIET	ALKALINE OR ACIDITY	DAYS ON DIET	ELECTRICAL REACTIONS IN MILLIAMPERES				AVERAGE	REMARKS
				C. C.	A. C.	A. O.	C. O.		
		cc. 0.1 N per 100 gm.							
Steenbock	6.65	530 alkaline	21	0.17	0.35	0.58	0.72	0.44	Rickets
Low phosphorus. Acid	0.90	350 acid	1	0.18	0.24	0.50	0.42	0.34	
			2	0.18	0.24	0.44	0.34	0.30	
			4	0.12	0.22	0.36	0.40	0.28	
Low phosphorus. Neutral	0.90	35 alkaline	1	0.16	0.20	0.42	0.48	0.32	
			2	0.14	0.24	0.50	0.44	0.34	
			4	0.18	0.32	0.60	0.64	0.44	
Low phosphorus. Alkaline	0.90	860 alkaline	1	0.04	0.10	0.20	0.10	0.11	Tetany
			1	0.08	0.16	0.34	0.30	0.22	Latent tetany
			2	0.14	0.18	0.40	0.40	0.28	
			3	0.18	0.32	0.64	0.56	0.42	
			5	0.16	0.28	0.52	0.52	0.38	
			7	0.12	0.20	0.50	0.70	0.38	
High phosphorus. Alkaline	0.50	860 alkaline	1	0.08	0.12	0.20	0.16	0.14	Tetany
			1	0.10	0.14	0.30	0.22	0.19	Latent tetany
			2	0.08	0.10	0.18	0.18	0.14	Tetany
			3	0.16	0.18	0.30	0.30	0.24	
			5	0.10	0.18	0.38	0.32	0.24	
			7	0.12	0.18	0.50	0.30	0.28	
Neutral	0.50	15 alkaline	1	0.10	0.14	0.26	0.16	0.16	Tetany
			2	0.18	0.22	0.70	0.44	0.38	
			3	0.18	0.28	0.66	0.52	0.42	
			5	0.16	0.32	0.74	0.62	0.48	
			7	0.18	0.20	0.78	0.56	0.44	
High phosphorus. Acid	0.50	350 acid	1	0.14	0.18	0.58	0.40	0.32	
			2	0.12	0.16	0.44	0.34	0.28	
			3	0.18	0.36	0.56	0.56	0.42	
			5	0.18	0.36	0.60	0.70	0.62	
			7	0.20	0.30	1.00	0.96	0.82	
			15	0.34	0.24	0.78	0.88	0.56	
Zucker + C. L. O.	4.73	109 alkaline	1	0.20	0.32	0.64	0.74	0.48	
			2	0.16	0.34	0.72	0.62	0.46	
			5	0.16	0.38	0.80	0.80	0.54	
			7	0.12	0.32	0.80	0.62	0.46	

TABLE 1—*Concluded*

DIET	Ca:P IN DIET	ALKALINE OR ACIDITY	DAYS ON DIET	ELECTRICAL REACTIONS IN MILLIAMPERES				AVERAGE	REMARKS
				C. C.	A. C.	A. O.	C. O.		
		cc. 0.1 N per 100 gm.							
Steenbock + C. L. O.	6.65	530 alkaline	4	0.28	0.42	0.98	0.88	0.64	
			15	0.22	0.30	0.80	0.86	0.55	Healing rickets
Steenbock + C. L. O. + irradiation	6.65	530 alkaline	4	0.62	1.21	1.76	1.40	1.26	
			5	0.20	0.38	0.68	0.88	0.54	
			6	0.20	0.40	0.76	0.92	0.58	
			9	0.22	0.34	0.88	0.82	0.56	
			11	0.18	0.30	0.74	0.78	0.50	Healing rickets
Steenbock + irradiation	6.65	530 alkaline	4	0.52	0.82	1.34	0.98	0.92	
			6	0.18	0.28	0.68	0.82	0.50	
			15	0.19	0.30	0.93	0.84	0.57	Healing rickets

The histological examination of the bones revealed complete healing after two weeks' treatment by the lower phosphate additions. At this time, the values for calcium and phosphorus had returned to normal (10). X-ray pictures showed healed rickets except in the groups receiving the larger amount of phosphate. In these there was only partial healing.

The animals on the alkaline high phosphate developed diarrhea and did so poorly that the experiment was discontinued on the seventh day. All the rest, except those which died in convulsions recovered and thrived.

The electrical reactions are given in table 1. When cure was accomplished by irradiation of the diet or by the addition of cod liver oil, or by both, the amount of current required to produce a response became temporarily elevated. This phenomenon was not observed when cod liver oil was added to the rickets-producing diet of Zucker. In these animals the reactions remained normal throughout.

When phosphates are given to rachitic animals there is produced not only a cure of the rickets but also a transient tetany. During attacks of tetany the amount of current required to stimulate the neuro-muscular response becomes almost vanishingly small. Between attacks of manifest tetany, latent tetany is present. This is evidenced by the smaller amounts of current required to cause muscular contraction. These lie between the extremely low values in tetany and those in normal animals. A protocol of such a case follows.

Protocol

1928

May 28. A 49-day-old male rat, 21 days upon the ricketogenic diet, Ca:P = 6.65, shows the following reactions: 0.22 (C. C.) 0.38 (A. C.) 0.68 (A. O.) 0.60 (C. O.) 0.47 (average). The animal is placed upon the same diet with sufficient phosphoric acid added to give a ratio of Ca:P = 1.0.

May 29. The animal is easily excited. Its electrical reactions indicate a lowered threshold of neuro-muscular irritability: 0.18 (C. C.) 0.16 (A. C.) 0.50 (A. O.) 0.32 (C. O.) 0.30 (average).

May 30. The animal, two days upon the new diet, has still lower electrical reactions: 0.10 (C. C.) 0.16 (A. C.) 0.20 (A. O) 0.24 (C. O.) 0.18 (average). This indicates latent tetany.

May 31. At 8:00 a.m. the animal is found in clonic convulsions, with carpopedal spasm. The electrical reactions are very low: 0.08 (C. C.) 0.08 (A. C.) 0.12 (A. O.) 0.12 (C. O.) 0.10 (average). This indicates that the condition is tetany.

At 11:00 a.m. the rat has recovered from tetany, and the electrical reactions are higher: 0.16 (C. C.) 0.28 (A. C.) 0.42 (A. O.) 0.54 (C. O.) 0.32 (average).

June 1. The animal appears normal, though easily excited. The electrical reactions are: 0.14 (C. C.) 0.30 (A. C.) 0.58 (A. O.) 0.64 (C. O.) 0.42 (average).

June 2. At 12:15 p.m. the animal is observed in manifest tetany. The electrical reactions are extremely low: 0.06 (C. C.) 0.08 (A. C.) 0.10 (A. O.) 0.16 (C. O.) 0.10 (average).

June 3. The animal has completely recovered and the experiment is discontinued.

The reactions for the high phosphate alkaline group remained moderately low and the reactions for the high phosphate acid group moderately high. The rest of the groups receiving phosphate showed normal electrical reactions within 2 weeks.

Discussion. From the above results it is clear that the mechanism of the cure of the defective mineral metabolism was accomplished by two essentially opposing methods, cod liver oil and irradiation causing temporary hypo-irritability, and phosphates temporary hyper-irritability. The acid-base factor of the phosphates though definite was of secondary importance.

If the response to galvanic stimuli is a measure of the physico-chemical environment necessary for muscular contraction, and more especially of the state of ionized calcium and phosphate in the blood serum, our results indicate that the drop in one case and the rise in the other are coincident with an increased calcium activity when cod liver oil or irradiated food is administered and an increased phosphate activity when phosphates are fed. The effect of the acid and base is that expected from studies of Hastings and Sendroy (11). Acid causes an increase in calcium activity and alkali a diminution. Alkalosis tends towards tetany and acidosis towards its cure.

SUMMARY

The response of rachitic rats to the administration of cod liver oil or irradiation shows a lessened irritability, and to the addition of phosphates, a heightened irritability of the neuro-muscular system. Acid phosphate additions cause a diminished, and alkaline phosphate diets an increased irritability. As measured by the galvanic current, the condition resulting from the addition of phosphates to the diet of rachitic rats should clearly be called tetany.

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STUDIES OF THE BRAIN STEM

XI. The Effects of Artificial Stimulation and of Traumatism of the Avian Thalamus

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The diencephalic portion of the brain has been made the focus of attention by many groups of recent workers in quite different fields. At least four such fields of interest may be cited: the physiological interest in the visceral and metabolic effects caused by hypothalamic lesions; the clinical and physiological interest in the mechanism of the extra-pyramidal motility disturbances; the psychological interest in the question of thalamic participation in sensory or emotional activity; and the anatomical interest of workers in the field of comparative neurology.

A review of the metabolic relations of the hypothalamus has been recently given by Sachs (1). The recent clinico-anatomical studies of the extra-pyramidal disturbances are too numerous to cite here (2). The recent paper of Bard (3) is a valuable experimental extension of the thalamic-sensory and pseudo-affective studies of Roussy (4), of Head (5), of Woodworth and Sherrington (6), and Bazett and Penfield (7). The extraordinary complexity of the anatomical nerve pathways connecting the inferior thalamic areas with striatal and mesencephalic centers is evidenced by the published studies of a large number of neurological workers.

A study of the anatomical, the experimental and the clinical literature of this subject indicates that thalamic functions may be divided into two groups, first the neo-thalamic functions dependent on the rich fiber connections with the cerebral cortex and which obviously are abolished by removal of the cortex; and second, the paleo-thalamic or intrinsic thalamic functions which are retained after the removal of the cortex.

It is well known from numerous studies of decerebration of a great variety of laboratory animals (with the possible exception of the monkey and certainly the few available studies of the decerebrate condition in man) that after the removal of the hemispheres anterior to the thalamus and recovery from surgical traumatism, the thalamus animal retains normal visceral activity, the ability to maintain normal body temperature, an apparently nearly normal control of striated muscle activity as evidenced by posture, walking, etc., and exhibits various types of pseudo-affective

responses to pain, hunger, etc. All of these are abolished or altered in birds and mammals by transection of the brain just posterior to the thalamus. The mechanism of this thalamic control is largely obscure. It is possible that this control is dual, primarily neurologic but in part indirect by hormone factors. The recent studies of Bard (3), of Cannon and his associates (8), (9), present experimental evidence confirming the older belief that the thalamus is a center which reflexly affects the sympathetic system as a whole. In thalamic control there may therefore be implicated the endocrine factors, particularly of the supra-renal glands, and possibly of the hypophysis, in the disturbances following thalamic lesions. Sachs (10) has reviewed the older literature and has himself studied the effects of electrical stimulation of the thalamic areas in anesthetized monkeys and cats. He concluded that there is a variety of *reflex* effects on various muscles and visceral organs; that the regions of the thalamus from which these effects may be elicited are those in which the large afferent tracts terminate, particularly the fillet and superior cerebellar peduncle, the median nucleus being found inert to stimulation.

Previous studies of the writer have suggested to him that the brain of the lower birds, such as the pigeon, may offer peculiar opportunities for the study of the basal cerebral nuclei and the extra-pyramidal structures. This is dependent on several factors: first, the relative absence of a complex type of cerebral cortex and cortical projection tracts such as occur in all mammalian brains; second, the facility with which various types of cerebral surgical operations may be carried out in the birds; and third, the fact that in the absence of the mammalian type of cortex the birds are homeothermic animals exhibiting highly developed types of muscular activity and control. This view is complicated by the present uncertain status of the homology of avian cerebral and thalamic structures with those of the mammals. (See Ingvar (11).)

The purpose of the present work was to seek information of the intrinsic thalamic functions, excluding thalamo-striatal relations, by direct stimulation of the exposed thalamus in the unanesthetized decerebrate bird. Several weeks were allowed for recovery from the surgical traumatism of decerebration and for the ensuing degenerations of the descending cerebral tracts. In this way it was hoped to avoid the effects of both anesthesia and the complicating reflex effects due to stimulation of the descending cerebral fiber tracts, and hence test the question of whether or not any essentially intrinsic thalamic functions could be revealed by direct artificial stimulation. We of course recognize the artificiality of this method of stimulating nerve centers which presumably in the intact animal are stimulated only reflexly or by the physico-chemical conditions of the blood or brain tissue itself. Such a study to reveal the most significant results must be correlated with the anatomic structures involved.

Hence a brief summary of the gross features of this complex part of the pigeon brain is included.

The general type of avian brain differs from the mammalian, in several respects. The cerebral cortex is relatively small and primitive in type, whereas the corpus striatum is relatively large and complex. The olfactory connections are largely reduced or wanting. The central connections of the optic, auditory and vestibular nerves are the most conspicuous structures of the medulla and mid-brain. Correlated with the absence of highly developed cerebral cortex is the absence of the lateral hemispheres of the cerebellum. Anatomically therefore the corpora striata and the mid-brain are the more highly developed parts of the pigeon brain. The thalamus is relatively small. For the purposes of the present report of experimental work, the thalamic nuclear masses may be divided into four groups. First, the very conspicuous nucleus rotundus, which has rich ascending and descending connections with the striatum and mid-brain. Second, the antero-dorsal nuclei with strong striatal connections, the functional value of which judging from the anatomical fiber connections will therefore be largely eliminated or reduced by removal of the cerebral hemispheres.[1] Third, the medial and hypothalamic gray matter surrounding and adjacent to the lateral and inferior borders of the third ventricle. Fourth, the group of somewhat smaller lateral nuclei related to the optic tract, with which this report does not deal. Groebbels (12) has given a valuable summary of the internal structure of the thalamus and mid-brain of birds.

The point that the writer especially wishes to emphasize is this, that in the absence of cortical and striatal nerve tracts by combined decerebration and degeneration of descending cerebral tracts, and in the absence of technical errors, any observed effects of stimulation must be indicative of the intrinsic thalamic or thalamo-mesencephalic functions. To this extent it was hoped to throw some light on the basal-thalamic factors involved in the primitive extra-pyramidal control. The present report deals with the results obtained by various methods of direct stimulation of the exposed thalamus of pigeons.

METHODS. The pigeons were decerebrated four to twelve weeks before stimulation of the thalamus was attempted. Care was taken in the decerebration to avoid traumatism of the thalamus other than that of cutting the cerebral peduncles just anterior to the thalamus. In a successful preparation, one month after the decerebration it will be found that, if the

[1] This is a consideration which does not seem to have been fully recognized by all students of experimental decerebrations. Since in the mammalian brain, the lateral, dorsal and anterior thalamic nuclei are principally centers of cortical and olfactory projection tracts, decortication or decerebration anterior to the thalamus will eliminate all the functional activity of these nuclei except for possible local reflex arcs.

dura mater has been left *in situ*, the cavity left by excision of the cerebral hemispheres will be filled by clear cerebro-spinal fluid. Especial care was taken to keep these birds in good condition. Several weeks after decerebration, the cranial cavity of the decerebrate bird was opened without anesthesia by simply cutting through the overlying skin and dura mater. In this way there is obtained a perfectly clear view of the antero-dorsal surfaces of the thalamus and optic lobes. Usually in these birds the third ventricle will be found to be dilated, presumably due to the atrophy of the rather large striatal tracts. It is therefore possible to directly stimulate the antero-dorsal or the intra-ventricular surfaces or to thrust the stigmatic electrode within the gray matter of the thalamus. In addition to electrical stimulation we have also tried the effects of applying cold or warmth to the thalamus, and mechanical and traumatic stimulation by puncture with a probe, or gross destruction with a cautery. In stimulating with cold, we have placed ice directly on the thalamus or within the third ventricle, have washed it with cold Ringer's solution or have applied a jet of cold air. For warmth stimulation, we have used either a jet of warm air or a stream of warm Ringer's solution at 48°C. If care be taken to avoid gross traumatism the experiments may be repeated on different days in the same decerebrate bird. After thalamic traumatism, the bird may exhibit a group of metabolic disorders leading to death, as is later described. In the measurements of body temperature, the thermometer was inserted into the cloaca as far as possible to get the maximum reading. In all cases the temperature readings are the maximum ones obtainable. This is an important detail. The variations of the body temperature of normal adult caged pigeons at rest are from 40.5° to 42.5°C., a range of normal variation that must be borne in mind in considering possible experimental changes.

Results. Protocols illustrating the effects of the various types of effects following thalamic stimulation are given below.

Electrical stimulation. The electrical stimulation of the dorsal surface of the thalamus caused chewing movements and contraction of the muscles of the neck with rotation of the head and circular movements of the bird. With stronger stimulation there is spread first to the wing muscles and then to the legs. The stimulation of the ventricular surfaces of the thalamus is followed by a characteristic elevation of the feathers of the neck and trunk and by shivering and jerking movements of the bird. Stimulation of the deep thalamic ventricular surfaces posteriorly causes movements of the eyes and constriction of the pupils, followed by sudden violent forced flying and circular movements. When the electrode is thrust into the deep posterior portion of the thalamus the largest single nuclues with which it might make contact is the nucleus rotundus. Its large size and its extensive mesencephalic connections suggest that probably these motor effects are due to its stimulation. In two birds the electrical stimulation was

followed by distinct elevation of the body temperature. This was associated with elevation of the feathers, shivering and muscular tremor, convulsive or forced movements. In two instances vocalization was induced when the electrode was thrust into the substance of the thalamus.

Pigeon 204

November 18.	Pigeon 204 decerebrated. Weight, 470 grams.
January 11.	In good condition. Body temperature, 41.5°C. Weight, 403 grams.
2:55 p.m.	Cranial cavity opened, no bleeding. A clear view of the thalamus. Stimulation with induced shocks using unipolar method. The electrode is a straight needle insulated except at the tip by capillary glass tubing. Stimulation of the right side of the third ventricle very near the dorso-medial edge of thalamus: contraction of the neck muscles, spreading after one minute of stimulation, to the wings.
3:00 p.m.	Explore the right wall of the ventricle with the electrode. Avoiding movements of neck and wings. Intense shivering after stimulation ceases.
3:10	Stimulate the anterior stump of thalamus at point of transection of cerebral peduncle. No response by the bird.
3:15	Stimulate the right dorsal area of the thalamus. Chewing movements. Head rotates toward the left and backward 180°. Feathers elevated. Circular movements.
3:20	The bird is standing quietly. Stimulate the right internal wall of the ventricle below the taenia thalami. The right foot is lifted with tremor and twitching of foot. Repeat with bird held in the operator's hand. Flexion of both legs.
3:40	Electrode thrust deep into the ventricle and backward. Quivering of both eyes and pupillary constriction. No bleeding. After the electrode was removed, forced flying and prolonged circular walking movements, first to the left and later to the right.
4:00	Body temperature, 40.5°. The bird appears all right except that the feathers are elevated and it walks in circles to the right. No vomiting or bleeding at any time.
January 12.	The bird has developed marked diuresis. Hyperpnea. Body temperature 44.2°C.
January 13.	The bird is dead.

Pigeon 201

November 16.	Decerebration of pigeon 201. Weight 303 grams.
January 10.	Body temperature 41.3°C. Weight 304 grams.
1:05 p.m.	Cranial cavity opened to expose the thalamus.
1:30	Stimulation with induced shocks using the insulated unipolar needle electrode. Electrode thrust into the dorsal mass of the thalamus. Vocalization and vomiting. Feathers erected. Vomited material is stained with bile. Strong persistent shivering. Defecation, three times.
1:50	Electrode thrust within the substance of the thalamus. Slight swaying and incoördinate movements of the bird.

2:05	Body temperature 42.5°C. Electrode placed deep within the third ventricle. Movements of eyes and pupillary constriction. Sudden violent movement of head and wings. Electrode removed. Violent distortions of head and neck. Sudden precipitous flight. Nystagmus of head and eyes. Hyperpnea. Unable to stand. Toes flexed.
2:30	Body temperature 42.8°C. Bird quiet, but unable to stand.
3:30	Body temperature 41.5°. Feathers depressed.
January 11.	Bird is unable to stand on feet. No polyuria. Temperature normal.

Pigeon 200

November 16.	Pigeon 200. Decerebration anterior to the thalamus. Very little bleeding. Weight 370 grams.
January 7.	Bird is in good condition. Weigh 350 grams.
3:00 p.m.	Temperature 42.5°. Cranial cavity is opened, no anesthetic. A band of tissue is found running transversely across the third ventricle in the dorsal part of the thalamus.
3:10	Stimulate this band with induced shocks, bipolar electrodes. Feathers of body are erected. The bird sways backwards and forwards in the long axis of the trunk. Teetering movements: stands swaying on the tips of the toes. Chewing movements.
3:15	Electrodes placed just within the ventricle. Quick circular movements to the left. Vomiting. Later, generalized muscular tremor and unsteadiness.
3:20	Stimulation repeated. Quick jerky movements of the entire body, trunk, legs and wings.
3:30	Temperature, 43.1°.
4:00	Temperature, 44.1°. Hyperpnea. Mechanical stimulation of the walls and floor of the ventricle with blunt probe. Circus movements to the left. Feathers elevated.
4:15	Electrodes put deep within the third ventricle so as to stimulate the inferior portion of the thalamus. Stimulation causes violent irregular movements, first backwards and forwards and then to the right and left. This is later followed by generalized tremor.
4:35	The bird is recovering, it can stand but at intervals there appear jerking movements of the head and generalized body tremor.
5:00	During the past 30 minutes there have been several urinations; 5 grams of clear watery fluid secreted.
7:30	A profound diuresis; 18 grams of water have been secreted in the past three hours.
January 8.	The bird has secreted 38 grams urine since 4:30 p.m., Jan. 7.
9:00 a.m.	Body temperature, 39.0°.

Cold and warmth stimulation. In five decerebrate birds we have applied a small piece of ice directly to the exposed thalamus. In one bird a jet of cold air was played on the exposed thalamus. In two birds a jet of warm air or of warm Ringer's solution was played against the thalamus. Protocols are given below. The body temperature reactions of these birds is plotted in figure 1.

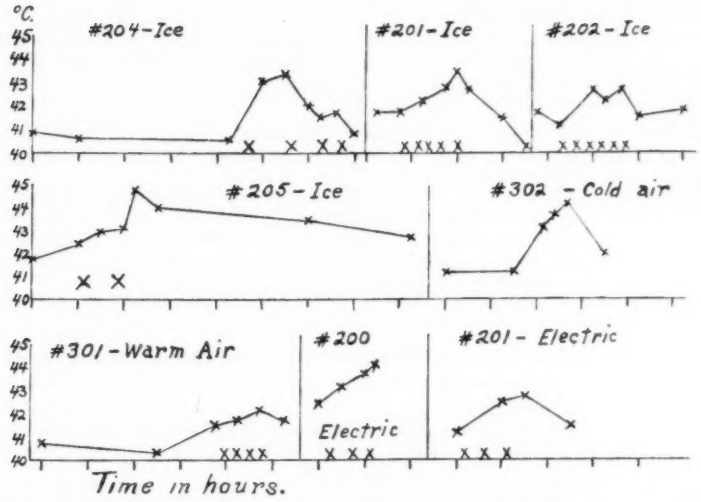

Fig. 1. Body temperature changes following direct stimulation of the exposed thalamus in 7 unanesthetized decerebrate pigeons by the various methods indicated. Body temperature readings in degrees Centigrade: time plotted in hours.

Pigeon 204

November 18.	Pigeon 204 decerebrated.
January 6.	Bird is in good condition.
9:30 a.m.	Temperature, 40.8°.
10:30	Temperature, 40.5°.
1:45 p.m.	Temperature, 40.5°.
2:00	Cranial cavity opened, no anesthesia.
2:05	A small piece of ice was placed within the third ventricle. Bird shivers; the feathers elevated.
2:10	Ice in ventricle. The bird snaps its beak repeatedly, otherwise it is standing quietly.
2:20	Ice put in ventricle. Bird stands quietly with feathers strongly elevated.
2:30	Body temperature, 42.9°.
2:45	Ice in ventricle. Occasional twistings of the head suggesting labyrinthine stimulation. Nystagmus-like movements of the head but nothing violent. Stands quietly on both feet.
3:00	Body temperature, 42.9°.
3:02	Ice to ventricle, immediately followed by a beautiful polypnea for one minute. Followed by chewing movements.
3:30	Temperature, 42.0°. Ice to ventricle. Polypnea.
3:35	Ice to ventricle. Polypnea follows.
3:37	Ice to ventricle. Polypnea.
3:45	Ice to ventricle. Bird stands quietly, no disturbances.
3:55	Temperature, 41.5°. Bird preens its feathers.

4:15	Feathers depressed.
4:25	Temperature, 40.8°. Experiment discontinued, bird seems to be in good condition. On January 11, the same bird was used for electrical stimulation of the thalamus.

Pigeon 201

November 16.	Decerebration of pigeon 201.
December 28.	Cranial cavity opened, no anesthesia. Bird is in good condition. Stands quietly preening its feathers.
2:20 p.m.	Temperature, 41.8°.
2:35	Ice to the ventricle followed by strong shivering.
2:40	Violent shivering, temperature rises to 42.5°.
3:00	Ice to ventricle.
3:05	Ice in ventricle.
3:10	Ice in ventricle. The bird is unable to stand. Strong, jerking, strychnine-like twitchings of wings and rump at intervals of about 10 seconds.
3:12	Bird stands on feet. Strong shivering and jerking movements. Bird falls, crawling circular movements to the left. There has been no bleeding at any time.
3:18	Temperature, 42.8°. Sudden violent flight. Flies in circles and directly into obstacles. Bird is held in hand, jerking movements continue. Feathers depressed.
3:25.	Ice to ventricle.
3:30	Temperature, 43.5°. Jerking movements of the entire body every 5 seconds. Unable to stand on feet. Feathers depressed, no hyperpnea.
3:45	The jerking movements are subsiding. Temperature, 42.7°. There has been no bleeding from the brain.
4:00	Tremor, bird unable to stand.
4:30	Bird is able to stand on feet, but unsteadily. Temperature, 42.0°.
5:00	Temperature, 41.1°. Jerking movements at intervals.
December 29.	Temperature, 40.1°. Bird seems normal, given feed and water. This bird was used at a later time for electrical stimulation of the thalamus.

Pigeon 301

December 5.	Decerebration of pigeon 301. Weight 500 grams.
March 6.	Bird is in good condition. Fasting weight, 515 grams.
11:45 a.m.	Bird quiet, temperature 40.8°.
2:15 p.m.	Temperature 40.2°. Bird sleeping.
3:30	Cranial cavity opened, no anesthesia. Temperature, 41.6°.
3:48	A stream of warm air is played on the exposed thalamus. This is continued for 20 minutes. Bird quiet, feathers depressed.
4:15	Temperature, 41.7°.
4:25	Thalamus is washed with warm Ringer's solution (50°C.).
4:35	Temperature, 42.2°.
4:45	Warm Ringer's solution to thalamus.
5:00	Temperature, 41.7°. There has been no vomiting, no shivering or muscular disturbances. Experiment discontinued, with bird in good condition. The next day the bird exhibited typical decerebrate behavior.

The application of ice or cold air was uniformly followed by a rise of the body temperature. This is preceded by erection of the feathers and by shivering which may be either mild or violent, associated with irregular jerking movements. In two instances when the temperature reached a level above 43°C., polypnea appeared. These results may be considered as confirmation of the work of Barbour (13) that the local application of cold to the cerebral thermogenic centers causes an elevation of the body temperature. Barbour however working with mammalian forms considered that this is a striatal mechanism, whereas in the present work the effects are clearly thalamic in origin. It should also be noted that similar elevations of temperature but less marked, with associated shivering, muscular contractions, hyperpnea and elevation of the feathers, may be elicited by electrical and traumatic stimulation of the thalamic centers.

TABLE 1

Water content of the blood and breast muscle of normal adult pigeons after various times of fasting and water deprivation

Water analyses were made in duplicate, the average of the two being given.

CONDITIONS	BODY WEIGHT	WATER IN BLOOD	WATER IN MUSCLE
	grams	*per cent*	*per cent*
Normal diet and water	310	80.95	75.24
Normal diet and water	265	82.03	74.65
12 hours, no feed or water	369	79.90	71.52
12 hours, no feed or water	323	77.48	71.51
12 hours, no feed or water	260	78.08	73.68
48 hours, no feed or water	300		70.32
48 hours, no feed or water	290		71.44
96 hours, no feed or water	260	80.21	71.82
96 hours, no feed or water	302	77.72	70.81
144 hours, no feed or water	248	77.93	70.32

Sachs (10) has emphasized the fact that with the most careful surgical technique and without infection, a post-operative rise in temperature always follows operative proceedings in this part of the brain.

Another striking effect of the electrical stimulation of the inferior thalamic area was a marked diuresis in the twenty-four hour period following the stimulation. As we have shown previously (14) this diuresis, if excessive, may be associated with elevation of the body temperature.

Diuresis following traumatic puncture, stimulation or gross destruction of the hypothalamic and infundibular areas has been so extensively studied by numerous recent workers that the literature need not be here reviewed. The observation of death following hypothalamic irritation, diuresis and temperature disturbances in some of the birds studied in the present series,

correlated with the writer's previous experimental studies suggested that actual tissue dehydration might be an essential cause of death. We have therefore repeated observations of the effects in pigeons of simultaneous decerebration and traumatism of the thalamus, and at the time of death have removed pieces of the striated breast muscle (which forms the principal mass of the pigeon's body) and determined its water content. Water was determined by drying a known weight of the freshly excised muscle for 48 hours in a constant temperature oven at 103°C. The water content of the muscular tissues of a series of birds which died following the onset of hypothalamic puncture polyuria, as compared with a series of water determinations of the same muscle tissue of normal pigeons on a normal diet and of normal fasting pigeons is given in tables 1 and 2. It is evident from these tabulated findings that in adult fasting pigeons, following traumatic destruction of the inferior thalamic region, the ensuing diuresis leads

TABLE 2

Water content of breast muscle of adult pigeons in which the thalamus had been cauterized within 24 hours preceding the death of the bird and the time of the analysis

Twenty-four hour fasting preceded the thalamic operation and no feed or water was given after the operation. Loss of body weight due principally to the loss of water by diuresis. Water determinations run in duplicate, the average of the two being given.

BODY WEIGHT AT TIME OF OPERATION	BODY WEIGHT AT TIME OF DEATH	WATER IN MUSCLE
grams	*grams*	*per cent*
300	273	69.44
325	311	68.75
367	330	70.76
275	234	69.02

to a marked dehydration of the muscular tissues. The constancy of the figure at the time of death, compared with the variability of the water content of the same tissues in normal and fasting birds, suggests that tissue dehydration is a cause of death of such animals.

SUMMARY AND CONCLUSION

The use of decerebrate pigeons, one to three months after removal of the cerebral hemispheres anterior to the thalamus, furnishes a method for the study of certain intrinsic paleo-thalamic or hypo-thalamic functions and for the observation of the effects of local stimulation of the exposed thalamus after the degeneration of the descending cerebral tracts and without the complications of anesthesia.

We have studied the effects of electrical stimulation, of the application of cold (ice and cold air), of warmth (Ringer's solution and warm air),

and of traumatic irritation by probe and cautery on the thalamus of the pigeon prepared in this way.

The effects of stimulation of the thalamus under these conditions may be divided into two groups; somatic muscular, and visceral or metabolic.

Electrical stimulation of the antero-dorsal area of the thalamus is followed by chewing movements and by slight muscular movements particularly of the neck with secondary circular movements of the bird. Electrical or mechanical stimulation of the inferior thalamic areas is followed by violent muscular movements of the neck, trunk, wings and legs, with forced, circular or convulsive movements. These are probably mediated through the nucleus rotundus, the largest nucleus of the inferior thalamus and one richly connected with the mid-brain and medulla oblongata. Weak electrical stimulation or the application of ice to the thalamus, is followed by shivering and irregular jerky movements of the entire body associated with a rise in body temperature.

The body temperature was increased by the application of cold and by electrical and mechanical stimulation of the thalamus, indicating that elevation of the body temperature may be the result of any type of irritation. However, in addition to this traumatic factor it was found that the application of cold was a much more efficient means of stimulating an elevation of the body temperature than the same degree of slight traumatism without the cold stimuius. Direct stimulation of the thalamus causes erection of the feathers, a generalized sympathetic effect. The body temperature is increased because of the concomitant sympathetic stimulation of the erector muscles of the feathers, with increased conservation of heat, and the stimulation of the somatic striated muscles to shivering, tremor and irregular jerking movements, with increased production of heat. When the body temperature had been increased to a certain level by the local application of ice to the thalamus, further stimulation by cold was followed by polypnea, a method of heat loss in the bird. We have not been able to cause a depression of the body temperature by local warming of the thalamus. Vomiting and defecation frequently followed thalamic irritation.

Mechanical, electrical and traumatic irritation of the inferior thalamic areas may be followed by severe diuresis, which persists in the fasting bird and leads to a real tissue dehydration which may be an essential cause of death in these birds.

In the experimental analysis of the primitive brain-stem mechanism of (extra-pyramidal) motor control in the birds, in addition to the primary anatomical structures involved, at least two metabolic factors must be considered, namely, body temperature and muscular tissue hydration alterations.

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ACTIVITY STUDIES ON CASTRATED MALE AND FEMALE RATS WITH TESTICULAR GRAFTS, IN CORRELATION WITH HISTOLOGICAL STUDIES OF THE GRAFTS

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Wang (1923) and Hoskins (1925) have demonstrated independently that the spontaneous activity of male and female rats is decreased permanently to a very low level by removal of the sex glands. In the more active female the decrease is often as great as 95 per cent; in the less active male it is usually about 65 per cent. The activity can be increased, however, to a permanent high level in the castrated male by successful implantation of ovaries (Wang, Richter and Guttmacher, 1925), and subsequent removal of these grafts has the same depressive effect as initial castration.

Normal males ordinarily are much less active than females, and do not show the four-day cyclic fluctuations which are such a striking feature of the female's activity. After the transplantation of ovaries to castrated males, definite effects of feminization can be detected in the activity curve. Practically all animals with successful grafts show an increase in activity to the higher level of the female, and, in some instances, the four-day cyclical changes. The activity of animals with unsuccessful ovarian grafts, on the other hand, remains on the flat low level characteristic of the castrates. It has not been possible to correlate the histological features of the graft with the increase produced in the activity, and attempts to explain the lack of correlation on the basis of differences in the vascular supply of the grafts have also been negative. Accordingly it has been impossible to determine what part of the ovary is essential for the production of activity.

The present study is concerned with the activity of castrated male and female rats with testicular grafts. As in the previous investigation, an attempt has been made by histological observation to correlate the survival of the grafts with the activity of the rats, and to determine by histological examination, if possible, the exact cellular elements which are responsible for the physiological "takes."

METHODS. Methods described in detail in previous papers were used to record and measure the activity in the present experiments (Richter and

Wang, 1926), (Richter, 1927). It will be sufficient to state here simply that each rat was confined in a cage consisting of a small living compartment and a revolving drum, so arranged as to allow the animal to pass freely from one to the other. In such cages every revolution of the drum in either direction is registered on a ratchet cyclometer, so that a simple objective measurement of the animal's spontaneous activity is obtained.

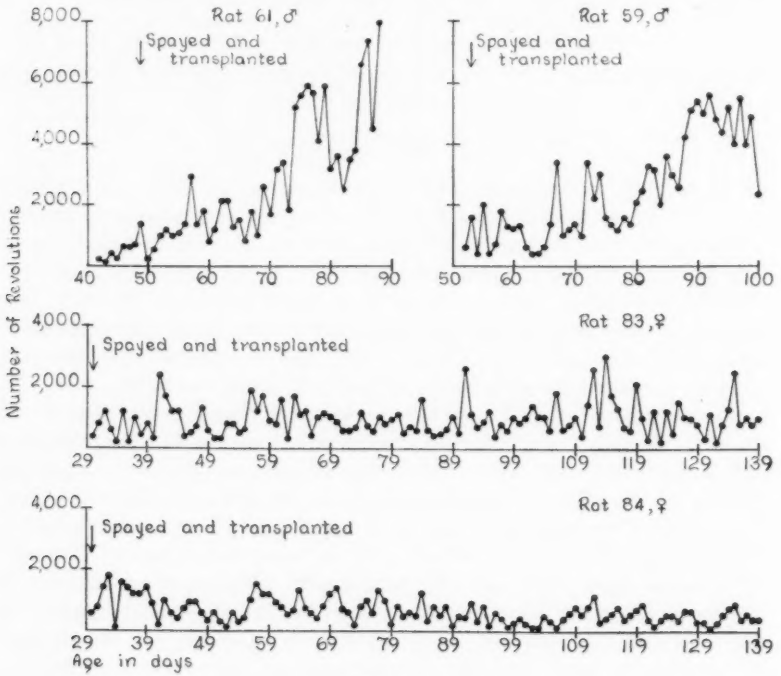

Chart 1

The methods used in the testicular transplantation and castration were very simple. The donors were usually only 3 or 4 days of age and never over 10 days, so that the transplanted testes were very small (about 2 mm. in length). The testes were implanted in the subcutaneous tissue of the walls of the scrotum in the males and in the recti muscles over the abdomen in the females. A few punctures were made with a fine needle through the dense tunica albuginea before the glands were implanted, and the skin against which they were to rest was scraped slightly with a knife. Then they were fastened into place by means of a single small piece of silk. Most of the transplantations were made when the recipients were very

young, and the animals were castrated at the same time. The ovaries were removed from the female through a small opening on each side of the back, just below the level of the ribs, after the tubes had been tied off with a piece of silk, and the testes were removed from the male through a small incision made in the tip of the scrotum.

In most instances the grafts were not removed for histological study until their effect on the activity of the animal was quite clear. After removal, they were fixed in Bouin's fluid and sectioned, and the sections were stained with hematoxylin and eosin and with Mallory's connective tissue stain. Several of the grafts were investigated for their viability by the injection of India ink to determine the extent to which they had established lymphatic connections, and several animals showing functional "takes" were stained vitally with trypan blue to determine the character of the cells comprising the increased interstitial tissue.

Results. The results of the present series of experiments are presented in table 1. Those animals which showed a definite permanent increase in activity following implantation of the testes are listed as "functional takes" (see chart 1, rats 61 and 59); animals showing slight sporadic bursts of activity above the castrate level are classified as "possible functional takes," (see chart 1, rat 83); and those showing no change at all are designated as "functional non-takes" (see chart 1, rat 84). It can be seen from the table that only seven of the thirty-six animals used are "functional takes." Since but two of these seven are females, it appears that testicular grafts are much less successful in females than they are in males. This fact is further confirmed when it is noted that in nineteen females there are only two "takes," (or slightly less than 10 per cent), while there are five male "takes" in seventeen (or 30 per cent). One female and one male are listed as "possible functional takes," and the remaining eleven males and sixteen females are "functional non-takes."

The percentage of "takes" on the basis of spontaneous activity is very small in comparison with that obtained from ovarian transplantation into males. It is important to note further that the increase in activity produced by transplanted testes is much smaller than that produced by transplanted ovaries. This is in keeping with the fact that normally the male rat is less active than the female. Presumably the testis in the rat is not as great a stimulus to activity as the ovary; hence transplanted testes elicit less activity response than ovarian grafts.

The results of the gross and microscopic examination of the grafts are also given in table 1. Well-preserved, easily-detected grafts were removed from all of the five male "takes" and from one of the two female "takes." In the other female, however, no trace of the transplanted glands could be found. It is very probable, therefore, that the relatively high activity level in this case was due not to the testicular transplant but to ovarian rem-

TABLE 1

	NUMBER	FUNCTIONAL RESULT	SIZE OF GRAFT AT TIME OF REMOVAL	ORIGINAL SIZE OF GRAFT	MICROSCOPIC TESTICULAR TISSUE	TUBULES	SERTOLI CELLS	SPERMATOGONIA	SPERMATOCYTES	SPERMATIDS	SPERMATOZOA	INTERSTITIAL TISSUE-CELLULAR HYPERTROPHY	INTERSTITIAL TISSUE-FIBROUS ACELLULAR	INTERSTITIAL TISSUE-CELLULAR INFILTRATION AND DETRITUS	TUBULES OF EPIDIDYMIS	DEGREE OF DEGENERATION
				mm.												
♂ Functional takes	59 ♂	"Good"	2 masses—10 mm.	2	++	+++	+++	++	+	+	0	+++			++	I
	61 ♂	"Very good"	2 masses—10 mm.	2	++	+	+	+	0	0	0	+++			++	I
	124 ♂	"Fair"	1 mass—5 mm.	2	++	+++	+++	++	++	0	0	+++			−	I
	127 ♂	"Fair"	1 mass—5 mm.	2	+	Tr.	0	0	0	0	0	0		++	−	IV
	126 ♂	"Fair"	1 mass—7 mm.	2	++	++	++	+	0	0	0	+++			−	I
♀ Functional takes	38 ♀	"Fair"	0	2	0	0						0				VI
	64 ♀	"Fair"	1 mass—4 mm.	2	++	+++	+++	+	0	0	0	+++			++	I
♂ Possible functional takes	31 ♂		2 masses—6 x 2 mm.	2	++	+	+	0	0	0	0	0	++	0	+	II
♀ Possible functional takes	83 ♀		1 mass—2 mm.	2	Tr.	Tr.	0	0	0	0	0	0	+	0	−	V

♂ Non-takes	30 ♂		2 masses—5 x 1 mm.	2	++	Tr.	0	0	0	0	0	0	++		+	IV
	32 ♂		2 masses—3 x 1 mm.	2	+	Tr.	0	0	0	0	0	0	++		+	IV
	60 ♂		2 masses—3 x 1 mm.	2	+	Tr.	0	0	0	0	0	0	+	+	–	IV
	62 ♂		2 masses—3 mm.	2	+	+	+	Tr.	0	0	0	0	+		–	III
	63 ♂		1 mass—2 mm.	2	+	+	+	0	0	0	0	0	+		–	III
	66 ♂		2 masses—4 x 2 mm.	2	+	+	+	0	0	0	0	0	+		–	III
	120 ♂		0	2	0										–	VI
	121 ♂		0	2	0										–	VI
	122 ♂		0	2	0										–	VI
	123 ♂		1 mass—6 x 5 mm.	2	++	+	Tr.	0	0	0	0	0	++	0	–	III
	128 ♂		1 mass—4 x 3 x 2 mm.	2	++	0	0	0	0	0	0	0	+	++	–	IV
♀ Non-takes	33 ♀		0	2	0											VI
	36 ♀		0	2	0											VI
	67 ♀		0	2	0											VI
	68 ♀		0	2	0											VI
	69 ♀		0	2	0											VI
	72 ♀		1 mass—6 mm.	2	+	+	Tr.	0	0	0	0	0	+		++	III
	73 ♀		0	2	0											VI
	74 ♀		2 masses—6 mm.	2	+	+	0	0	0	0	0	0	+	+	+	IV
	86 ♀		0	2	0											VI
	129 ♀		0	2	0											VI
	130 ♀		0	2	0											VI
	131 ♀		1 mass—2 x 1 mm.	2	Tr.	+	+	0	0	0	0	+	0	0	–	III
	132 ♀		0	2	0											VI
	134 ♀		0	2	0											VI
	135 ♀		0	2	0											VI
	136 ♀		0	2	Tr.	+	+	0	0	0	0	+	0	0	–	III

nants left when the animal was spayed.[1] Small masses were found also in the two "possible takes" and in twelve of the "non-takes." The grafts were apparent but greatly degenerated in eight of the eleven male "non-takes," whereas traces of transplanted gland were found in only four of the sixteen female "non-takes." In the remaining three males and twelve females the grafts had been entirely absorbed.

It is apparent from these data that testicular grafts are not nearly so successful in the females as in the males. How can this fact be explained? Moore (1924) has shown that the testis is deleteriously influenced by an increase in temperature above that prevailing in the scrotum. In our experiments the grafts were implanted beneath the skin over the recti muscles of the abdomen in the females, whereas they were implanted in the scrotum in the males. According to Moore and Quick (1924) the temperature in the scrotum is about eight degrees lower than that in the wall of the peritoneal cavity. A greater number of successful transplants in male rats, therefore, was to be expected.

Examination of our material has shown both grossly and microscopically that the actual "takes" are associated on the whole with grafts which are in a far better state of preservation than those found in the "non-takes." Furthermore, observations of the size of the grafts when implanted, and their size when removed, demonstrate that the testes continue to grow very materially, particularly if they become functional "takes." However, increase in size alone is not a sufficient criterion of a functional graft. Combined with increased size, there must be a survival of the cells constituting the testes, and more especially, according to our data, a survival of the interstitial portions of the organ. Four out of five male "takes" show a very definite growth of cellular interstitial tissue, while one at least contains viable cells of some description. In the "non-takes," on the other hand, the interstitial tissue, when present, is of a highly degenerated, acellular, fibrous character. In one group of "non-takes," to be sure, it appeared from gross examination that there had been some growth of the transplants. Microscopic study reveals, however, that the increase in size is due to an entirely acellular, fibrous type of dead tissue comprising the interstitial tissue of the glands. The growth of the grafts in the group of functional male "takes," we believe, is due to proliferation of the specific elements of the glands, and is not attributable to a mere infiltration of a degenerating testis by phagocytic cells. We were able to demonstrate this fact conclusively by an experiment in which we stained two rats

[1] Since this animal was spayed, we have learned that unless considerable care is exercised when the tubes are tied, a small remnant of the ovary may be left in the body. This subsequently hypertrophies, with the occasional result that the animal may run a great deal and show a reaction very different from that which follows complete removal of the ovaries. (See Wang and Guttmacher, 1927.)

vitally with trypan blue before their grafts were removed. On subsequent study of these two grafts we found that the majority of the cells constituting the interstitial tissue had not stained vitally. Hence we knew that the cells of this tissue did not belong to the category of macrophages or clasmatocytes, which are very abundant in a fibrous zone of encapsulation

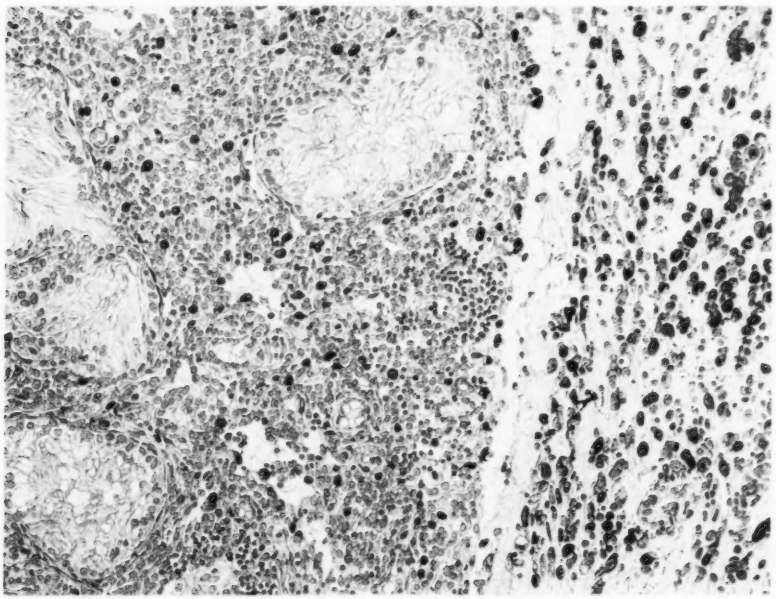

Fig. 1. Drawing of a portion of a grafted testis in rat 61, ♂, ×220. This is a typical picture of a graft which has become a functional "take" (activity record shown in chart 1). In the gross this testis measured 10 mm. in diameter and had grown from an original size of 2 mm. at the time of transplantation.

In the picture a portion of the periphery of the graft is shown. The tubules are markedly degenerated, possessing only Sertoli cells and in a few instances spermatogonia. The interstitial tissue is tremendously hypertrophied and cellular. Moreover the cells constituting the hypertrophied interstitial tissue appear to consist of specific testicular elements and not of infiltrated blood or wandering cells. The rat had been stained vitally with trypan blue before the graft was removed and the result can be plainly seen. The macrophages or clasmatocytes which are vitally stained are shown as cells possessing a deep black, somewhat vacuolated, or granular cytoplasm. A tremendous number of wandering phagocytic cells is seen in the encapsulating connective tissue. The number of vitally stained macrophages in the interstitial tissue, on the other hand, is not greater, but even less than one would normally find there.

We wish to express our appreciation to Mr. James F. Didusch for this illustration.

enclosing the graft, but are present in no more than normal numbers within the gland itself (fig. 1). We believe, therefore, that in the functional "takes" the transplants have increased in size by actual hypertrophy; i.e., by an absolute increase in the amount of specific cellular interstitial tissue.

It has been possible to demonstrate by a second method that the "functional" grafts have established themselves. In two of the animals with "functional takes" india ink was injected into the graft in situ. The ink thus injected escaped from the grafts through lymphatics into the nearest inguinal lymph nodes in one instance, and into abdominal nodes, in the other. We have repeated this experiment on several of the grafts of the non-functional type, and in every instance we have failed to fill the lymphatics in this manner.

The changes overtaking the tubular elements of the testes are more variable. The tubules are never functioning fully, but show every grade of degeneration from the slightest to the most complete. In the group of functional "takes" the tubules are best preserved, showing almost invariably the presence of Sertoli cells, spermatogonia and spermatocytes, with occasional spermatids, but never spermatozoa. In the female "non-takes" the tubules are usually in a state of complete degeneration, while in the male "non-takes" they have fared slightly better, showing, in a few cases, the remains of Sertoli cells.

On the whole the evidence seems to indicate that the interstitial tissue determines the survival and functional value of the grafts. A number of instances might be cited from the non-functional males and females (table 1) in which the mass left contains some degenerate tubules possessing viable Sertoli cells, but no viable cells within its interstitial tissue.

The epididymis has been examined in several animals, and it has been found that the tubules of this organ survive longer than those of the testis proper, and that they may persist occasionally in a fair state of preservation in testes otherwise highly degenerated.

The summary of microscopic data is presented in table 1 as follows. The grafts have been rated by number on a scale ranging from one to six, (1 representing maximum preservation), determined by the different degrees of degeneration within the graft, with regard to both tubular and interstitial elements. The degrees of preservation of the individual elements in the testes have been represented by symbols: tr = trace, + = slight amount, ++ = moderate amount, +++ = large amount. Only one other point in the table requires explanation. Interstitial tissue has been subdivided into three categories: 1, interstitial tissue which is cellular and increased in amount, probably functional; 2, interstitial tissue which is fibrous and acellular, and is probably non-functional, and 3, interstitial tissue which is composed largely of detritus and shows a marked cellular

infiltration. In some instances this type may possess slight functional capacity.

REMARKS. It occurred to us that a testicular graft might have some effect on the reproductive tract of the female, and that this effect might be detected in the vaginal smears. Such an effect, if detected, would present an excellent opportunity for a study of periodic changes in the testes, in the same way that the periodic changes in the ovaries can be studied.

The smears of twelve females with testicular transplants were studied over a period of months. In several instances the smears occasionally showed cornified cells, which are not present in ordinary spayed females. However, so far as we have been able to determine up to the present time, the appearance of these cells had no visible relation either to the spontaneous activity of the animal, or to the histological structure of the graft. Nevertheless, it may be possible, with this method and with more successful transplants, to study changes in the function of the testes.

SUMMARY

1. The activity of castrated male and female rats, which is much less than that of normal rats, can be increased very considerably by implantation of testes.

2. Testes were implanted into thirty-six animals, seventeen males and nineteen females, Six of the males and two of the females listed as "functional takes" showed a definite increase in activity; one male and one female listed as "possible functional takes" showed a doubtful increase; and all of the remaining animals listed as "functional non-takes" showed no change at all in the activity level.

3. Large grafts were found in all of the animals showing a definite increase in activity, with the exception of one female which probably had not been fully spayed. Small grafts were found in the two "possible takes," and in eight of the eleven male "non-takes" and four of the sixteen female "non-takes."

4. Microscopic study revealed that the testicular grafts were not absolutely normal in any of the animals. In general it can be said, however, that the increase in activity stands in direct proportion to the state of preservation of the graft, and that the most important part of the testes for the production of the activity increase appears to be the interstitial tissue.

5. Testicular transplants "take" much better in males than in females.

6. The increase in activity produced by transplanted testes is much less than that produced by transplanted ovaries.

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THE EFFECTS OF BICARBONATE BUFFERS AND OF CARBON DIOXIDE ON THE MOTOR FUNCTIONS OF THE EXCISED SMALL INTESTINES OF RABBITS

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Bicarbonate buffer systems, although inferior to the phosphate buffers in regard to the maintenance of the hydrogen ion concentration, are greatly superior from the standpoint of maintaining the rhythmic motor activity of the intestines, as was pointed out by Rona and Neukirch in 1912. It appeared desirable to study in some detail the relation of these effects to the concentration of the bicarbonate, especially within the physiological range, for peristaltic as well as for pendulum movements; and to endeavor to determine the share of the hydrogen ion concentration, of the bicarbonate ions, and of the carbonic dioxide or carbonic acid in these effects. For this purpose, the same arrangements were used as described in our preceding paper (Sollmann, von Oettingen and Ishikawa, 1928) as also, the same Locke solution, namely, per cent, NaCl = 0.9; KCl = 0.042; $CaCl_2 \cdot 2H_2O$ = 0.024; dextrose = 0.1. We may first describe the phenomena in the long intestines, and then use the Magnus intestines for the experimental analysis.

THE EFFECTS OF BICARBONATE ON THE PERFUSED LONG INTESTINE. In a series of experiments, the entire small intestines of rabbits were suspended in Locke solution, the pH being kept at 7.6 by the periodic addition of n/100 NaOH; their lumen being perfused by the same solution. The technic of the method has been described by von Oettingen, Sollmann and Ishikawa, 1928. Under these conditions, i.e., in the absence of bicarbonate, the intestines lengthen rapidly by fall of tonus and the peristalsis becomes progressively weaker and irregular, so that the perfusate flows passively instead of being actively propelled, as shown in the beginning of figure 1A. When 0.03 per cent of $NaHCO_3$ was added the striking improvement illustrated by figure 1B takes place: the tone rises markedly; peristaltic waves appear and become strong and regular; the pendular movements also improve; the outflow becomes propulsive instead of passive. This improvement was lasting, figure 1, C and D. If Na_2HPO_4,

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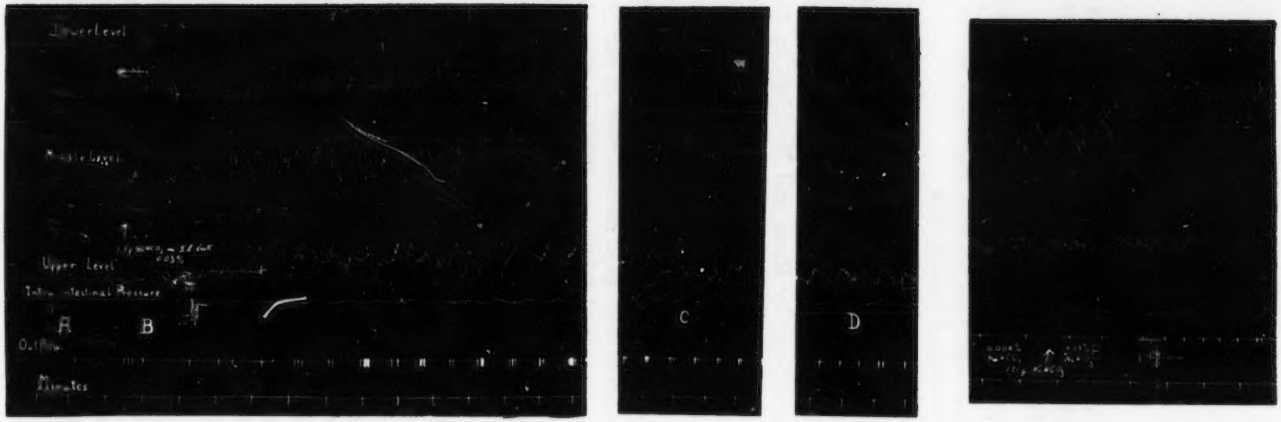

Fig. 1. Effects of sodium bicarbonate on the movement of the excised entire small intestines of the rabbit, suspended in and perfused by Locke solution. The three upper curves are the myographic tracings of different level of the intestines; the fourth tracing is of the intraintestinal pressure changes at the lower (cecal) end; the fifth records the propulsion of the liquid through the intestines, the outflow being signalled by a dipping bucket; the lowest line is the time, in minutes. The tracing starts with Locke's solution kept at pH 7.6 by the periodic addition of NaOH. At B, 0.03 per cent sodium bicarbonate was added; C was taken fifteen minutes and D one hour later. E is from another experiment, and shows the effects of increasing the bicarbonate concentration. The intestines had been in Locke solution buffered with 0.008 per cent bicarbonate for an hour and a half, the pH falling gradually from 7.7 to 7.2, by the formation of fixed acid with gradual destruction of the bicarbonate. At the arrow, 0.03 per cent of bicarbonate was added, raising the pH to 7.8.

0.024 per cent or 0.048 per cent was added in place of the bicarbonate, there was also marked stimulation, but the changes were not as favorable as with the bicarbonate, the movements were much less regular, the tone-rise less marked, and the activity declined earlier.

As was shown in our previous paper (Sollmann, von Oettingen and Ishikawa, 1928), the quantity of bicarbonate required to maintain the pH of the Locke solution within the physiologic range depends on the degree of aeration; with the lively aeration of the Magnus arrangement 0.008 per cent is optimal, but with the long intestines, the more sluggish aeration of the larger tank requires 0.03 per cent of bicarbonate. Accordingly it was found that movements of the Magnus segments continue in 0.008 per cent bicarbonate practically undiminished for over three hours; but that the perfused intestines in the large bath soon decline in this concentration of bicarbonate, but are well maintained if the initial concentration was 0.03 per cent, as shown in figure 1D. Thus, in three experiments with 0.008 per cent bicarbonate, the pH of the bath sank from 7.6 to 7.2 in half an hour. The peristaltic activity diminished notably within fifteen to twenty minutes, so that the circular contractions became relatively feeble and traveled sluggishly if at all, and the original active, peristaltic propulsion of the perfused fluid was changed to a passive, continuous flow. Within an hour the peristaltic contractions had generally disappeared altogether, leaving only the pendular activity. Increasing the concentration of the bicarbonate to 0.03 per cent, promptly improved the tone, the vigor of the contractions and the propulsion (fig. 1E).

It remained to be seen whether the deterioration of the long intestines in 0.008 per cent bicarbonate is due merely to acidification; or whether the peristaltic movements which predominate in the long intestines require the stimulus of a higher concentration. In the next series of three experiments, therefore, the bath was started with 0.008 per cent bicarbonate, and the pH of the bath was restored from time to time by the addition of n/100 NaOH. This was found to maintain the peristaltic activity much better; sometimes almost perfectly for three hours; generally, however, the peristalsis deteriorated gradually within one and a half to two hours. This could be due to the loss of bicarbonate, through the liberation of CO_2 by the partial acidification between the periodic additions of the alkali. In the next series of three experiments, the bicarbonate was restored by neutralizing the acidity with bicarbonate instead of NaOH. This was found to maintain the activity as well as the original addition of 0.03 per cent of bicarbonate. It may therefore be concluded that the deterioration of the perfused intestines in the large bath with 0.008 per cent bicarbonate is due to the loss of bicarbonate; and that 0.008 per cent of bicarbonate is ade-

quate, for peristalsis as well as for pendular activity, provided that the loss of bicarbonate and the changes of pH are prevented.

There remained the question whether concentrations of bicarbonate higher than 0.008 per cent further improve the peristalsis of the perfused intestines. To answer this, the concentration of bicarbonate was increased by successive additions of 0.008 per cent, at intervals of about ten minutes, restoring the pH to 7.6 by N/10 HCl. This would decompose a part of the added bicarbonate, but not all. Five experiments gave generally concordant results: In the absence of bicarbonate with the pH adjusted by NaOH, the movements become very weak and irregular and the flow is passive (fig. 1A). With the first addition of bicarbonate, strong peristaltic waves arise, the tone improves and the propulsion becomes active. With the second addition, the peristalsis may be further improved; or the intestines may tend to become spastic. With further additions, this spasticity increases as indicated by prolonged systole or double peaks, imperfect relaxation, and diminished propulsion. Increase of bicarbonate concentration therefore tends to stimulate the circular muscle to increase of peristalsis or to spasticity. The optimal concentration for peristaltic propulsion lies between 0.008 and 0.016 per cent. In practice, however, 0.03 answers quite well and has the advantage that it furnishes a reserve of bicarbonate, sufficient to maintain adequate bicarbonate concentration and constant pH for several hours.

It may be concluded then, that sodium bicarbonate increases the tone and peristaltic activity of the perfused intestines, proportional to the concentration of the bicarbonate, within the limits investigated. It need not follow from this, however, that the effects are due to the bicarbonate ions, for alterations in the bicarbonate concentration also entail changes of the hydrogen ion, of carbonate, and of carbonic acid and carbon dioxide concentration, and these are further complicated by the aeration. It was shown, however, that the responses to bicarbonate differ from those of equivalent changes of pH produced and maintained by sodium hydroxide, or by phosphate; so that it appears that the hydrogen ion concentration is not the cause of the characteristic bicarbonate effects.

THE INFLUENCE OF THE CONCENTRATION OF BICARBONATE ON THE MAGNUS SEGMENTS, WITH RELATION TO THE HYDROGEN ION CONCENTRATION. The further differentiation of the effects of bicarbonate from hydrogen ions and carbon dioxide was attempted on Magnus segments, because of the greater convenience in changing the solutions. For this purpose, the response of these segments to increase of bicarbonate was studied under three conditions of pH, which serve to contrast and thus to neutralize the adventitious factors: In the first series, the increase of pH produced by the bicarbonate was left uncorrected; in the other series, the pH was readjusted by means of HCl or NaOH immediately after the addition of the bicarbon-

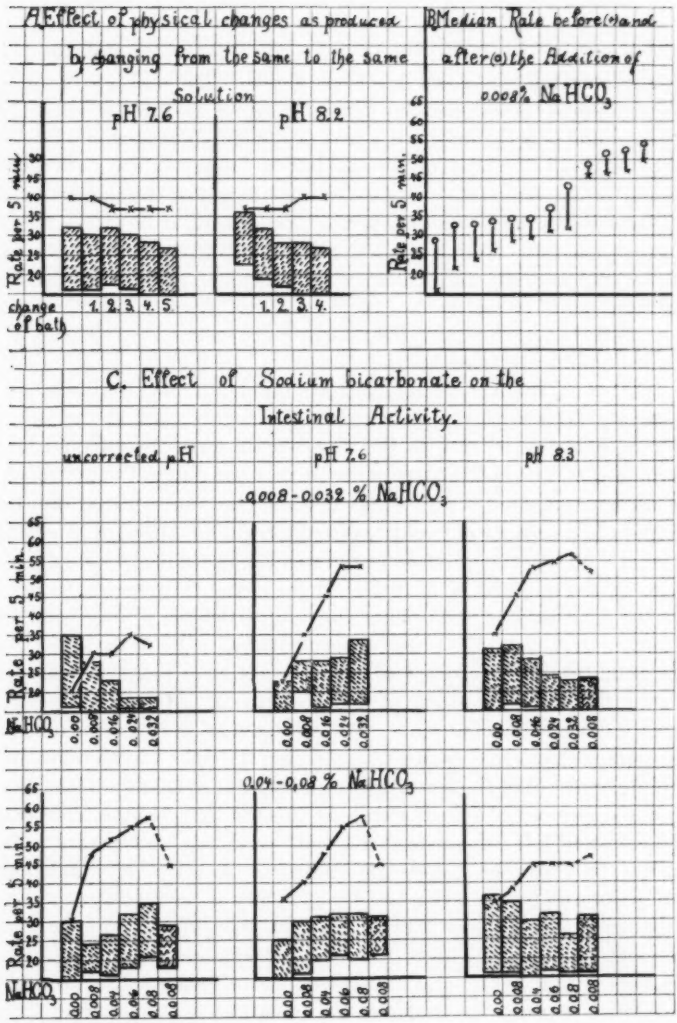
A. Effect of physical changes as produced by changing from the same to the same Solution
pH 7.6
pH 8.2
Rate per 5 min.
change of bath
1 2 3 4 5
1 2 3 4
B. Median Rate before (o) and after (o) the Addition of 0.008% NaHCO3
Rate per 5 min.
C. Effect of Sodium bicarbonate on the Intestinal Activity.
uncorrected pH
pH 7.6
pH 8.3
0.008-0.032 % NaHCO3
0.04-0.08 % NaHCO3
Rate per 5 min.
NaHCO3

Fig. 2. Medians illustrating the effects of increasing the bicarbonate concentration on Magnus segments of rabbit's small intestines. The line graph corresponds to the rate, the blocks to the excursions and tone. Increasing concentrations are shown by solid lines and simple hatching, decreasing concentration by broken lines and cross hatching. The medians were taken from the tracings shortly before changing the bath.

A. Exchange of fluid in the bath without change of composition.

B. Relation of the degree of 0.008 per cent bicarbonate acceleration to the initial pendular rate.

C. Increase of bicarbonate concentration, with pH uncorrected, and with adjustment at 7.6 and at 8.3.

ate, to 7.6 in the second series, and to 8.3 in the third series; thus eliminating hydrogen ion changes as a factor in these series. The addition of the bicarbonate increased the bicarbonate concentration in all three series, although not to an equal degree; for at 7.6, a part of the bicarbonate would be decomposed; and at 8.3, the alkalinity would convert a part of the metabolic carbonic acid into bicarbonate. In all the series, however, the changes of bicarbonate ions would be in the direction of the amount of bicarbonate added. The other carbonic factors need not be considered; for the dissociated carbonic acid resolves itself merely into hydrogen and bicarbonate ions; the concentration of carbon dioxide is equalized in all the mixtures by the lively aeration; and the concentration of undissociated H_2CO_3 and of CO_3 ions is presumably insignificant.

In brief, then, the bicarbonate ions are the only factor that changes in a constant direction with the addition of bicarbonate; and if, therefore, the functional changes are due to the bicarbonate ions, they should also be uniform in direction in the three series; if they are due to the hydrogen ion, their direction should vary. The matter was investigated by taking tracings from segments of freshly excised intestine suspended in a Magnus bath, first with bicarbonate-free Locke solution (with dextrose) adjusted to pH 7.6, by the addition of n/10 NaOH (or in the third series, to pH 8.3). When the contractions had become constant, 0.008 per cent of bicarbonate was added, and then the other concentrations, with intervals of ten to fifteen minutes. Four groups of experiments were made in each series, changing the concentrations of bicarbonate in the various sequences between 0.008 and 0.08 per cent. The results of all the experiments of each group were then charted as medians showing the rate, and the systolic and diastolic excursions of the lever, before and after each change of bicarbonate. The simple increase of bicarbonate was effected by adding a more concentrated solution; but for constant pH and decreasing concentrations it was necessary to exchange the entire solution. A series of blank control experiments was first made in the same manner as the regular experiments, but replacing the solution with an identical solution, five or six times in succession. Figure 2A shows that these exchanges do not produce any alterations. The figure also illustrates the extent of the spontaneous changes that occur in the course of the experiments. They were insignificant at 7.6; at 8.2 there was a progressive loss of tone. It may also be premised that the phenomena with decreasing bicarbonate concentrations are so nearly the reciprocals of those of increasing concentrations, that only the latter need be included in the medians, which are shown in figure 2C.

It will be seen that all the charts, in all three series, show a uniform effect on the rate of the pendular contractions, which accelerates progressively with increase of the bicarbonate concentration. It may also be noted that the rate curves are parabolic, the acceleration becoming progressively

smaller for successive additions. This is explained by the observation that the degree of acceleration for a uniform concentration of bicarbonate (0.008 per cent) is inverse to the natural rate, as shown in the group of twelve experiments, in figure 2B. It is a manifestation of the Weber-Fechner law of diminishing returns in proportion as biologic reactions approach their maximum or completion. This complemental summation of the bicarbonate stimulation and natural rhythm is evidence that both involve the same mechanism (Barlow and Sollmann, 1926).

The changes of amplitude, tone and periodicity phenomena appear more variable because they are partly reciprocal to the rate changes and they may also involve qualitative alterations which cannot be averaged. Their medians therefore appear confused, as in figure 2C, and the phenomena must be studied on the individual tracings. This also makes it possible to take account of the numerous variations of conditions which were introduced in the experiments, but which were either excluded or extinguished in the medians. These variations included the use of segments that had been kept on ice, as well as segments excised from the living animal; gradually progressing and abrupt changes of the bicarbonate concentration; addition of the bicarbonate to the bath and change of the bath, with more or less modification of the osmotic concentration of the bath, in both directions, but always by negligible amounts, i.e., less than a hundredth of the original molecular concentration; and finally, the three conditions of pH, namely, uncorrected, and constant at 7.6 and at 8.2 or 8.3. Notwithstanding these differences in conditions, the results are entirely consistent and qualitatively similar. The only important difference is that with the alkaline reaction of pH 8.3 depression sets in more rapidly, especially when the intestines are over-stimulated by the higher concentrations of bicarbonate. The changes of the individual phenomena with increasing concentrations of bicarbonate from 0.008 to 0.8 per cent may be summarized from the tracings as follows:

The *rate* increases progressively in the three series, confirming the medians. The *amplitude* varies: It generally increases with the rate, on adding 0.008 per cent bicarbonate to the unbuffered Locke solution. With further increase of the bicarbonate individual experiments may progressive increase of amplitude; but in the majority the amplitude tends to decrease reciprocally to the rate, but not in simple ratio. The decrease is least if the pH is kept at 7.6 and greatest if it is kept at 8.3, especially if the intestine is at the same time overstimulated by the highest concentration of bicarbonate (0.08 per cent). The series with uncorrected pH is intermediate. *The diastolic tone*, i.e., the reciprocal of the length of the segments at the trough of the contraction waves, tends to rise with increasing concentration of bicarbonate, more rapidly above 0.03 per cent. This is most marked in the pH 7.6 series, least in the 8.3, and intermediate with the uncorrected pH.

Periodicity, i.e., rhythmic variations in the amplitude of the pendular movements or rhythmic tone changes analogous to peristaltic contractions in form and in tempo, is slight or absent in 0.008 per cent bicarbonate Locke solution; but if it is present, it increases in rate and less markedly in strength when the bicarbonate approaches 0.03 to 0.04 per cent, and these concentrations may sometimes start periodicity if it was absent. Above 0.04 per cent, the periodic rhythm may again become slowed. Once started, it tends to persist even if the segment is transferred to 0.008 per cent of bicarbonate. The periodicity stimulation is again most marked in the pH 7.6 series, and least marked in the 8.3 series.

It appears advisable to illustrate these descriptions by a few typical tracings. Figure 3A shows the increase of rate, amplitude and tone on adding 0.008 per cent of bicarbonate to Locke solution, which had been adjusted to the same pH (7.6) by sodium hydroxide. This happens to be from an iced segment.

B shows the progressive increase of tone, rate and periodicity in 0.024 per cent bicarbonate, in the same segment of intestine, at the same pH (7.6).

C: a fresh segment, illustrates the progressive increase of tone and periodicity of the more common type, on increasing the bicarbonate from 0.008 to 0.04, 0.06 and 0.08 per cent and again at 0.008, all at pH 7.6. Above 0.04 the periodic rhythm becomes slowed, but it quickens and persists in 0.008 per cent. The continuous line between the tracings corresponds to the original diastolic tone.

D: a fresh segment, shows the increase of rate and amplitude, without change of diastolic tone, on increasing the bicarbonate from 0.008 to 0.04 and then to 0.06 per cent, with pH uniform at 8.3.

The study of the individual tracings therefore confirms that the changes of amplitude, tone and periodicity, as well as the rate, are essentially the same in the three series, notwithstanding the different courses of the pH. This indicates that the stimulation is due to the bicarbonate ions; their influence being sufficiently strong to overshadow any effects that the pH may tend to produce in these concentrations.

From a practical standpoint, it may be added that, although the stimulant effect of bicarbonate increases with the concentration, as high as tried (0.08 per cent), the concentration of 0.008 per cent is preferable for the Magnus bath, because it automatically maintains the pH at a more favorable level. These data and conclusions are a confirmation and extension of those of Rona and Neukirch, who also found that bicarbonate improves the contractions, even when the pH is kept constant by the addition of HCl. The reactions of the cat's intestine seem to differ in some details from those of the rabbit, for according to A. W. Young (1914) increase of bicarbonate from 0.01 to 0.04 per cent increased the tone without changing

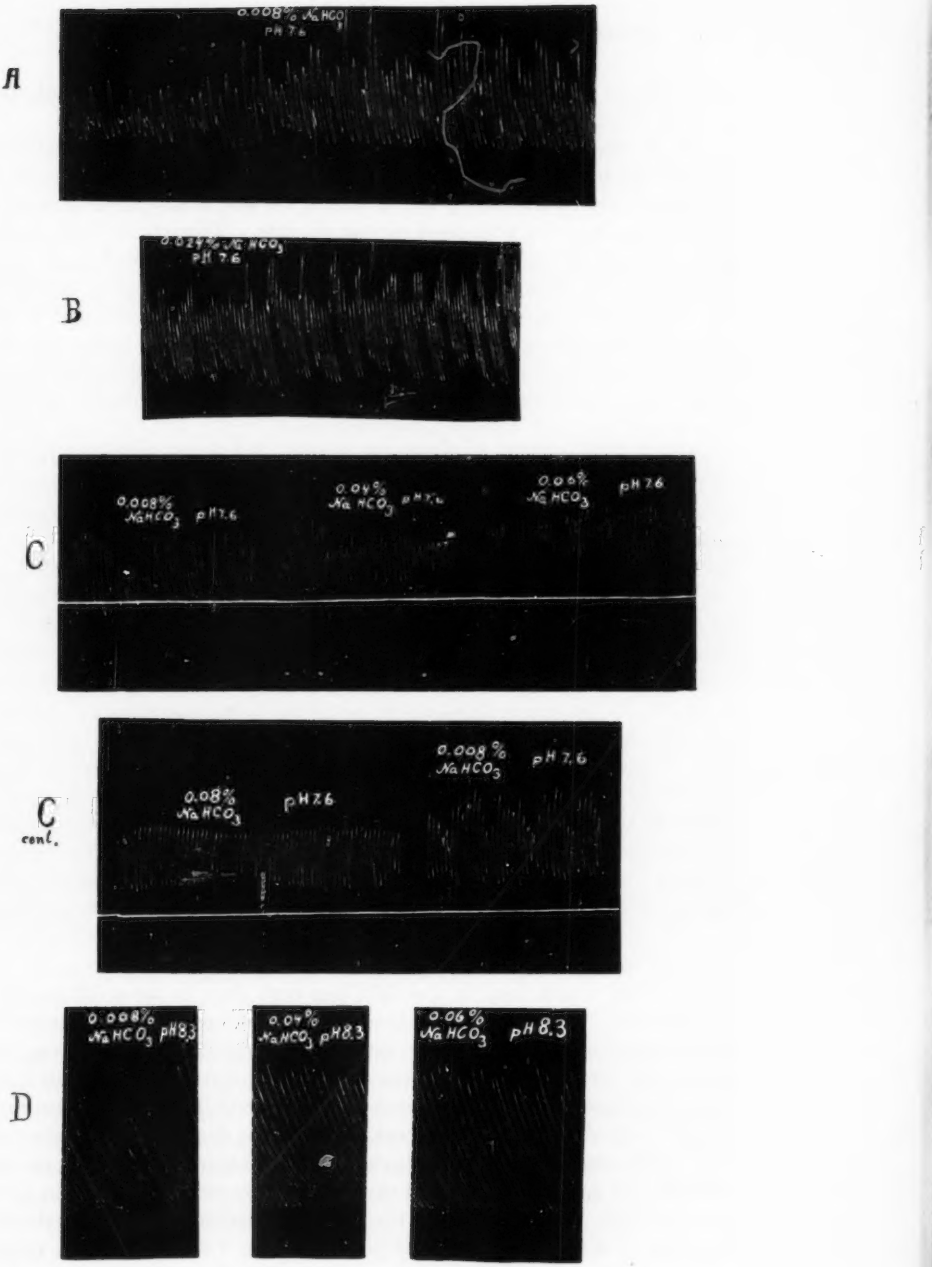

Fig. 3. Type tracings of the effects of increasing bicarbonate concentrations on Magnus segments of rabbit's small intestines.

the pendular movements. Higher concentrations diminished both tone and amplitude.

THE INTER-RELATION OF THE EFFECTS OF CARBONIC ACID AND BICARBONATE ON THE MAGNUS INTESTINE. Since bicarbonate and carbon dioxide are converted into each other by alterations of the pH, it appeared desirable to inquire also into the actions of carbon dioxide. These have been studied quite extensively. As may be seen from the review of Lois Fraser (1925) the carbon dioxide appears to be purely depressant to Magnus intestines; the effects agreeing qualitatively with the corresponding changes of pH, although quantitative differences from other acids, in the degree and time relations, have been described, and attributed to differences in penetration. Since then Polansky (1927) has confirmed that the effects of carbonic acid on perfused intestines are also depressant.

Although there is this general agreement as to the depressant effects of carbonic acid, it appeared desirable to differentiate further between the share that the increased hydrogen ion concentration, the carbon dioxide as such, and the decreased concentration of bicarbonate ions may have in the depression, especially within the more physiologic range, between 8.3 and 7.0. A separation of carbon dioxide from the other factors was first sought by comparing the effects of uniform concentrations of carbon dioxide when added to Locke's solution containing 0.008 and 0.03 per cent of sodium bicarbonate, with the thought that the effects of carbon dioxide as such would be the same in both solutions, while the more concentrated bicarbonate solution would protect more perfectly against reduction of pH and of bicarbonate ions. The carbon dioxide concentration was adjusted either by adding definite proportions of a saturated solution of carbon dioxide in 0.008 or 0.03 per cent Locke solution, or more satisfactorily by aerating the solutions with oxygen containing five per cent (volume) of carbon dioxide. By the first method it was found that when the concentration of carbon dioxide is higher than one-tenth saturation, the acidity rises so much even in the 0.03 per cent bicarbonate that the intestinal movements are promptly arrested. With one-tenth saturation, however, the depression is always very much less in the 0.03 per cent than in the 0.008 per cent bicarbonate. The carbon dioxide content would be alike in both solutions (5.55 volume per cent at the moment of mixing); the pH, however, was materially lower in the 0.008 per cent bicarbonate (5.7 calculated, 6.0 observed) than in the 0.03 per cent bicarbonate (6.25 per cent calculated, 6.3 per cent observed). The same difference in degree of depression was seen, as illustrated in figure 4, when the Locke bicarbonate solutions were aerated with carbon dioxide and oxygen (5.95 by volume), which in about seven or eight minutes lowered the pH of the 0.008 per cent bicarbonate from 7.6 to 6.4; and of the 0.03 per cent from 7.8 to 6.8. The experiments were conducted by starting an intestinal segment in one of the

solutions, aerated first with air, and then with the carbon dioxide and oxygen; then transferring to the other solution and aerating at the same rate. The fact that the same concentration of carbon dioxide which depresses in 0.008 per cent bicarbonate does not depress in 0.03 per cent bicarbonate shows that the depression is not due to the carbon dioxide as such (in this range); there remain the hydrogen ion concentration and alteration of bicarbonate content. Figure 4A shows that on passing CO_2 into bicarbonate Locke solution, the alteration of the pH from 7.8 to 7.0

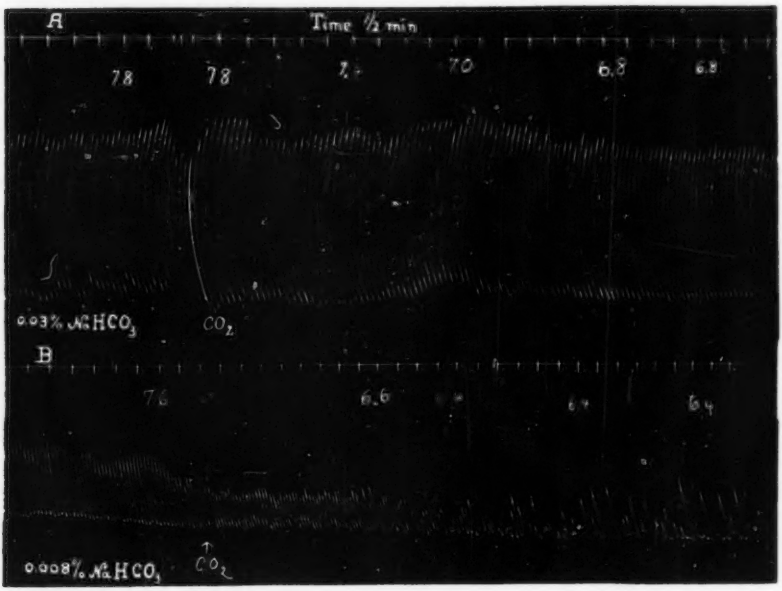

Fig. 4. Magnus segment in A 0.03 and in B 0.008 per cent bicarbonate, aerated with carbon dioxide, five per cent volume in oxygen.

produces very little effect, and even at 6.8 the depression is but slight, and figure 4B illustrates that it becomes more marked at 6.6, and progresses to 6.4; arrest of movements generally occurs between 6.2 and 6.0, although some segments still contract feebly at 6.0, the lowest point to which the pH was pushed. Essentially the same phenomena were observed when the Locke solution was buffered by 0.075 per cent of glycocoll, as suggested by Rona and Neukirch. When added to Locke solution made up with ordinary distilled water, this gives the pH of 7.0 to 7.2, which is well maintained with the intestines in the Magnus arrangement, rising gradually to

to 7.4 in three hours. The contractions are generally quite fair, usually of the pendular type, but sometimes with more or less periodicity. Extensive and varied comparisons were made between Locke's solution buffered with this percentage of glycocoll, with 0.008 per cent bicarbonate, and with the two combined, applied successively to the same segments and to different segments; and also with 0.024 per cent Na_2HPO_4. The responses were so similar that they cannot be said to differ more than do different experiments in the same buffer. The depression produced by acidity is therefore due to the hydrogen ions, and the removal of bicarbonate ions plays only a minor rôle. Comparative experiments were also made between HCl and CO_2, with the bicarbonate, with glycocoll and with 0.048 per cent Na_2HPO_4 buffers. Again the motor depression was practically indistinguishable in the three buffers. As to the two acids, the HCl appeared more often depressive above 7.0, but this was probably due to imperfect mixing, and possibly also to conversion of bicarbonate ions to CO_2.

Fig. 5. Carbon dioxide on the movements of intestines suspended in a moist chamber.

Between 6.4 and 6.0 the depression appears equally pronounced with both acids; for instance at 6.0 the proportion of segments that were arrested or practically arrested was 3/6 for HCl into 0.075 per cent glycocoll; 9/17 for CO_2 into 0.075 per cent glycocoll; and 9/15 for CO_2 into 0.008 per cent bicarbonate. The data as to CO_2 agree very well with those of Lois Fraser, who found that this arrested the strips between pH 6.4 and 6.2; with HCl, however, she did not obtain arrest until 5.2 to 4.6 and even lower. We do not know whether the difference is ascribable to the Fleisch solution which she employed, or to some other experimental factor. In any case, it may be concluded that the depressant effects of carbon dioxide are due essentially to the excessive hydrogen ion concentration.

CARBON DIOXIDE GAS ON THE CONTRACTIBILITY OF THE INTESTINAL SEGMENTS SUSPENDED IN THE MOIST CHAMBER. It appeared interesting to study also the effects of carbon dioxide by another method, namely, by allowing the gas to act on the intestines suspended in a moist chamber,

instead of being immersed in a solution. The conditions are probably not different in principle, because in the moist chamber the cells are also bathed in a film of liquid or lymph. The quantitative relations of CO_2, H_2CO_3, HCO_3 and CO_3, however, would probably be different, and it was conceivable that this might result in differences in the response. However, the CO_2 produced exactly the same depressant effects when it was added as gas to the moist chamber as to the bath (fig. 5). This agrees with the relaxing effects of carbon dioxide observed by Hooker (1912) on frog's intestines suspended in the moist chamber. Since movements of mammalian intestinal segments in the moist chamber have not hitherto been recorded, so far as we know, it may be added that the experiments were performed by suspending a 2 cm. piece of rabbit's intestine in the usual Magnus arrangement, except that the 50 cc. bath chamber was empty save for a shallow layer of 10 per cent dextrose solution, to moisten the oxygen which entered through the bottom of the chamber. The addition of 5 per cent volume of carbon dioxide to the oxygen causes marked depression and sometimes arrest; 25 or 50 per cent arrest promptly, but the movements generally recover completely in oxygen.

CONCLUSIONS

The addition of sodium bicarbonate in Locke's solution produces stimulation, manifested in Magnus segments especially by acceleration of the pendular rhythm, while in the lumen-perfused intestines the improvement of the peristaltic rhythm is more prominent. The stimulation increases with the concentration, at least to 0.08 per cent, and tends to pass into spasm. Practically, 0.008 per cent is sufficient to maintain good function, if it is present continuously; but with limited aeration it is better to start with 0.03 per cent. The bicarbonate stimulation is due to the bicarbonate ions, and independent of the pH; for it occurs also when the latter is kept constant.

The depressant action of carbon dioxide is not due to the dissolved CO_2 as such, but to the acidification; for it is materially less, for equal concentrations of dissolved CO_2, if the hydrogen ion concentration is kept above 6.8.

Changes of pH (within the range of 8.2 to about 6.8) or the addition of carbon dioxide within this range, have but little effect on the intestinal movements. Serious depression begins about 6.4, and arrest generally between 6.2 and 6.0.

The degree of depression by acidification appears about the same whether it is produced by CO_2 or by HCl, and whether the solution is buffered by bicarbonate, by glycocoll or by phosphate.

The effects of carbon dioxide on intestinal movements in the moist chamber correspond to those in Locke solution.

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DIFFERENTIAL RELAXATION DURING READING, WRITING AND OTHER ACTIVITIES AS TESTED BY THE KNEE-JERK

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The purpose of the present studies was to enquire whether in human subjects activities like reading or writing may go on even if the individual is at the same time so relaxed that tests reveal a diminished knee-jerk; that is, whether moderate activity in some muscle-groups nevertheless may permit a certain relaxation in others. Obviously general relaxation is not here in question, for with completely relaxed arms and eyes and other parts, such activities would cease; accordingly, if any degree of relaxation is to be possible under the conditions, it must be *differential*, a term later to be defined. In a previous investigation, Jacobson and Carlson (1925) found that when the individual lies down in advanced general relaxation, the knee-jerk reveals a diminution. This test of relaxation therefore was available to apply during various activities.

More specifically, we found, as clinicians well know, that when the average individual is requested to relax, the jerk is commonly increased. The tested limb often seemed to be held rigidly by the patient, restraining movement at the knee-joint, until he was requested to relax or to turn his attention from the hammer by looking up at the ceiling or out of the window. It seemed evident, as is commonly believed and as Hoffman (1918) has proved, that this release is due in part at least to relaxation of the muscles that oppose the knee-jerk, that is, chiefly the hamstrings. But our interest was in the effect of extreme relaxation of the general skeletal musculature on the knee-jerk: when this was brought about in subjects who (excepting A. J. C.) had received special training to relax, the jerk was diminished, often to the vanishing point. This result has been confirmed, according to Tuttle and Williams (1925), in their investigations where they produced general relaxation by excessive heat due to electric current of high frequency. In the writer's experience since 1925, the knee-jerk has proved to be a useful clinical test as an aid to determine whether a patient during practice at relaxation has reached an advanced stage, and diminution of the jerk under such conditions has been frequently observed.

No study of the present type, so far as I know, has been previously undertaken. Differential relaxation, although not known by that name, is frequently taught by the use of exercises in university departments of oratory, singing and pianoforte. The same is true of esthetic and ballet dancing. Delsarte pointed out to his fellow students in France the importance of such relaxation in sculpture and other arts. Accordingly it seems of interest to enquire whether we can find any foundation for these views and practices of artists.

METHODS. The present investigation was made with 40 subjects of whom 21 were medical students, 5 were individuals in a markedly nervous condition and 14 were subjects who had received special training to relax by a method previously described (Jacobson, 1924).

During the tests on all subjects, the attempt was made to render the conditions favorable to relaxation: Walls of celotex building-board were installed so that the laboratory room would be fairly sound-proof. Noises were so far as possible avoided by the experimentor, excepting when he gave instructions. Since it was not unusual to find an increase of the knee-jerk appear upon approaching the subject or moving before him, the experimentor also tried to keep out of sight much of the time.

Apparatus served three purposes:

1. *To strike the tendon*, the spring hammer of Lee and Kleitman was used in the tests first performed, viz., series 3; but the stroke was increased to about 5 cm. In all other series the automatic electromagnetic hammer of Johnson (1927) was substituted, but to prevent slight slipping, due to its own forces upon impact, we were obliged to secure it with special clamps. At the outset of each test-period, an attempt was made so to adjust the hammer and so to gauge the strength of blow that a marked jerk would be produced. We were not certain that the initial jerk was maximal with any particular subject, but probably in the majority of instances it was nearly maximal.

2. *To record the amplitude of jerk*, the rod and lever system of Johnson was employed at first (series 3), but in later tests the string and pulley system recently described (Jacobson, 1928). We secured linear graphs whose lengths in centimeters are recorded in the present article. Much effort was expended to prevent possible shift of position of the knee during a period of tests, since the hammer striking at a different spot might thereby effect an alteration of extent of jerk: it was feared that the thigh might slip as the individual relaxed. Various measures failed to restrain this part until we finally strapped it firmly to a side-board attached to the right arm of the Morris chair occupied by the subject. In addition, the knee was pressed firmly against the side-board by an adjustable metal lath placed on the left. After each jerk, the leg was brought back to its former resting position by its own weight. These matters have been given in detail in the previous reference.

3. *To record the position of the knee*, testing whether in spite of all precautions there was any slipping, required a second kymograph. Forward deviation of the knee affects a frog muscle lever into which are soldered metal rods 3 mm. in diameter. The rear arm is 12.5 cm. long, but 2 cm. from the fulcrum it is bent down at a right angle, while at its tip is soldered a thin metal strip, 3 × 3 cm., covered with adhesive tape to protect the skin from irritation. One flat surface of the strip rests against the upper portion of the patella where it is not fastened in any way because the weight of the forearm of the lever keeps it in proper position. Including a writing-point, the length of the forearm is 63 cm. Evidently the effective length of the rear arm is measured from the fulcrum to the end of the strip, approximately 10 cm., yielding a magnification somewhat more than six-fold. Adjustments of position of the lever are possible, for the attached rod, which extends horizontally, is clamped at a right angle to a second rod which extends upwards to a horizontal board secured to the arm of the chair. Accordingly, when in place, the metal strip presses lightly against the patella and moves forward or backward with the knee, causing a rise or fall of the writing-point. While it is not necessary to attach the metal strip to the skin with adhesive, we did so in a few earlier tests with a certain lever which we soon discarded because it failed to magnify sufficiently.

Another lever serves to record lateral deviation or rotation of the thigh, magnifying somewhat more than two-fold. The distal arm is 29 cm. long, while the proximal arm, bent down at a right angle 2 cm. from the fulcrum, extends in all 14.5 cm., including a metal strip attachment, 3 cm. × 2.2 cm. This is firmly secured with adhesive tape to the inner aspect of the thigh a few centimeters behind the patella. Accordingly the writing-point of each of these levers makes a vertical tracing with each knee-jerk; but thereafter, provided that the knee does not shift, each lever returns to its former position of rest. If no shift takes place during the entire period, each lever (apart from the vertical tracings) will draw a fairly horizontal line. As standards for comparison, base-lines can be drawn with either lever at the beginning of the test period, turning the drum 360° by hand.

PROCEDURE AND RESULTS. *Untrained subjects. Series 1, 21 normal students* of both sexes were tested during periods of 40 to 70 minutes. Of 27 test periods, the subject continuously copied from a magazine in 17, wrote numbers to the beats of a metronome in 5, made an ergograph tracing with the right hand in 2, read aloud in 1, conversed actively in another, while in 1 instance no occupation was furnished. During writing, the instruction was given to proceed at a rate which seemed possible to continue for an hour. A more satisfactory occupation was writing numbers from 1 to 99 inclusive, then repeatedly, to the beats of a metronome. This was found to hold the attention better than the other occupations (at least

when the rate was made rapid) and permitted us to secure a constant rate of mental work throughout the period, if desired, or to vary the speed at will. We found the speed of writing numbers during a 2 minute period before and after each session with each subject and from this determined the average rate of speed per minute. It was then possible to set the metronome at a rate well below this speed, if desired, so that uniform writing could be secured throughout a period.

No request to relax was made; in fact, as a rule the word "relaxation" was not even mentioned and the purpose of the investigation remained unknown to the subjects. Taps to the patellar tendon were delivered every 30 seconds throughout the entire period of test without intermission, excepting to 8 subjects. For the latter, periods of 5 to 15 minutes occurred during which they continued at their task but with no stimuli from the hammer. In almost all instances, 24 out of 27, the jerk tended to decrease progressively following the initial strokes. This result was not as a rule influenced by the presence of 5 to 15 minute intermissions when no stimuli were delivered to the patellar tendon. The only effect after such a period was a still further drop in the extent of the knee-jerk.

It is of interest to plot the averages for the first 50 jerks of 13 subjects engaged in reading or writing, as shown in figure 1 (unbroken line). The curve has been twice smoothed by the method of averages, using five points at a time, but the original points are indicated. We have what closely resembles an exponential curve, showing the diminution of neuromuscular tension as time proceeds. If the reciprocal of the above 50 values is multiplied by 10 and plotted (fig. 1, broken line), a curve is secured which shows the extent of relaxation. Changes in the character of the curve near $X = 0$ and $X = 25$ can be neglected, since they are due to inadequacy of the method of smoothing near these points. The curve as a whole is to be compared with that of the learning process as noted by Bryan and Harter (1897) and Thorndyke (1923). Just as we can in a very general way speak of a curve of fatigue (Kronecker, 1871; Mosso, 1888) and of a curve of memory (Ebbinghaus, 1885), so we can speak of a curve of relaxation. Evidently the characteristics will vary greatly with individuals, their particular state of nervous hypertension or hypotension, the nature of the occupation and of the distractions present, the length of time seated before the first stimulus occurs, the strength of blow and various other factors.

We conclude that normal individuals engaged while seated in physical and mental tasks involving no direct activity of the leg muscles and no emotional excitement tend to become relaxed during the course of an hour as measured by the knee-jerk. To use a phrase common in biological literature, it is a process of "adaptation," whereby the organism, at first using neuromuscular energies not essential to a task soon omits them, so that more approximately only those energies which are required come to be in use.

Light is thrown on the above rule by the exceptions (3 out of 25). One subject (R. S.) when copying from a magazine or writing numbers to the metronome at a rate well below her greatest speed had shown characteristic diminution of the jerk as discussed above, at times almost to the point of disappearance. At such times she reported that she felt "relaxed" or "drowsy"; one could with care observe a difference in her facial expression and attitude in the direction of greater relaxation, most clearly in her forehead which was markedly wrinkled at the outset but became smooth later. Accordingly, in order to test whether the apparent relaxation was brought on by the relative ease and unexciting character of the task, during the next period a companion of hers was permitted to be present during the entire period so that she might converse spontaneously and interestedly. When this was done, practically no diminution of the jerk took place. Another test was made while writing numbers with the metronome rate well below her maximum speed, which was attended by diminished knee-jerks: a change to a high metronome rate, stimulating her to speedy writing, produced a change in the direction of heightened knee-jerk. At the new rate, however, after a series of relatively high jerks, the height tended again to decline, although not as a rule to the low level previously attained with the slower writing. A second exception occurred with subject I. L. J., while using the ergograph. Here the diminution of the jerk was but slight. It is readily understood that lifting a weight repeatedly will tend to have a reinforcing effect; accordingly we should anticipate that at least with some individuals little or no diminution of the jerk would take place under such conditions. The third exception occurred with a male medical student engaged in writing. No fall of the jerk took place: indeed the tracing is distinguished by high jerks that are unusually uniform to the end. Such uniformity is not in our experience characteristic of neurotics (see below); and the subject affirmed that he was not of nervous temperament. The explanation doubtless would have to be sought in further study of the habits and reactions of this individual.

Series 2, neurotic patients with no training to relax were tested under the same conditions. They, like the foregoing subjects, were not requested to relax, with one exception noted below. No psychosis was present in any of these and no organic pathology, so far as revealed by clinical investigation. Three out of 5 failed to exhibit diminishing jerks (fig. 2). This result contrasts with that obtained with most of the normal untrained subjects. Observation of the behavior of these three suggested that they failed to relax, as indeed the knee-jerk test indicated.

Two of the neurotic subjects appeared to relax during the activities. Of one the first ten jerks averaged 4.41 cm. on the graph; after a 10 minute interval free from stimulation, the second ten averaged 3.21 cm. After another 10 minute interval free from stimulation, he was requested to relax

so far as he knew how. He later reported that after a period of experience of hypertension ("he felt as if he would blow up"—"his right leg felt tense"), he tried to let go naturally, became interested in what he was reading, and seemed to relax. Fourteen jerks of this period averaged 1.61 cm., considerably less than those of his first series. Such diminution was not due to displacement of the hammer at the patellar tendon, for the position of stroke was finally changed 4 times without affecting the low type of jerk.

The other neurotic individual who appeared to relax complained of marked fears when he tried to recite in his classes and had shown a marked knee-jerk at the time of a general clinical examination; but during the present tests, he appeared undisturbed, with a relatively low jerk from the outset, while subsequently there was a diminution.

These studies indicate that even neurotic individuals may relax at certain times, with or without request, although not previously trained to relax. This finding does not conflict with the thesis that as a rule the neurotic disposition is characterized by habitual hypertension or by frequent hypertensive responses not exhibited by normal individuals. The present neurotic individuals, although able to relax to the extent indicated by the tracings, nevertheless apparently in their daily lives failed to use and apply such ability, as their clinical histories abundantly disclosed. Evidently they could and did relax upon some favorable occasions at least; but they were not habitually relaxed.

Trained subjects. Series 3,4,5. Of 14 trained subjects, 3 (C. H., H. S. and E. J.) were normal in their previous nervous history, while 11 had been patients with medical or nervous hypertensive disorders or both; but these patients had been previously discharged from treatment excepting 2: M. F., epileptic, still in the earlier stages of treatment; H. K., with previously spastic esophagus and nervous hypertension, recovered except for occasional moderate relapses. All had received training to relax lying down (general relaxation) and all but 3 (M. F., H. S. and C. H.) had received training to relax in the sitting posture (differential relaxation), but there had been considerable variation in the periods of training and in the apparent skill attained. Excepting two (D. M. and E. J.), no special training to relax while reading had been given.

Throughout the entire period the subject read aloud or copied from a magazine, being instructed to write as quickly as seemed reasonably possible for a prolonged time. The order of record was generally as follows: 1, a set of jerks "before relaxation" occurring irregularly from about ½ to 2 or 3 minutes apart during a period usually about 10 minutes (rarely as brief as 3 minutes or as long as 28 minutes); 2, an interval (usually 10 or 15 minutes, rarely as brief as 3 or as long as 17 minutes) to relax, during which the occupation was continued but the tendon was not struck; 3, a set of jerks "during relaxation" occurring irregularly from about ½ to 3 or 4

minutes apart (occasionally longer, up to 9 minutes) during a period on the average about 20 minutes, with extremes of 5 to 36; 4, usually but not always an interval of 3 to 5 minutes to cease relaxing, during which the occupation was continued but the tendon not struck; 5, a set of jerks "after relaxation" with conditions similar to the preceding. Occasionally the above was followed by another interval of 5 minutes to relax, a second set of jerks "during relaxation," a briefer interval to cease relaxing, and a final set of jerks "after relaxation."

After we had taken most of the records in this series (which was performed before *series 1* and *2*), we found in the last nine that the tendon could be tapped regularly every 30 seconds during a test period without making it impossible to relax. It is true that such relatively frequent blows tend to disturb relaxation and for this reason are disadvantageous, tending to destroy the very condition whose presence it is desired to register. But this difficulty is inherent in the knee-jerk as a test of relaxation and therefore we hope in future to find a better measure. However, we adopted and used during the remainder of the present work the 30-second interval because of the advantages of uniformity, relative frequency of test, continuity of curve, and the possibility of using an automatic hammer.

Series 4 was performed with 5 of the above trained subjects, but the occupation was writing numbers to the beats of a metronome as previously described. In this series the taps on the tendon were always 30 seconds apart and generally no intermission of the taps took place during or preceding the relaxing period. Levers as above described were used in this series, but the graphs so secured will be discussed later along with similar ones with untrained subjects.

Typical curves of the knee-jerks before, during and after relaxation periods for various trained individuals are shown in figures 3 and 4. As will be seen, the average jerk generally is decreased during relaxation as compared with the preceding period and often also as compared with the succeeding period, but relations are more clearly brought out in table 1. Here we are to compare arithmetic mean values (A. M.) for groups of knee-jerks before, during and after relaxation. But the successive knee-jerks of groups for any individual often differ greatly from each other in extent, while the A. M. of two groups compared with each other may differ much or little. Therefore we need some criterion to determine what difference between A. M. values should be considered significant rather than due to chance. Accordingly the standard deviations and probable errors are calculated in the usual manner $\left(\sigma = \sqrt{\frac{\Sigma v^2}{n}};\ \text{P. E.} = \frac{.67\sigma}{\sqrt{n}}\right)$. The ratio of the difference between the A. M. values of adjacent groups for each individual (for example, before and during relaxation, or during and after relaxation) to the sum of the probable errors of the same groups is shown

in the columns headed "R." Where $R \geqq 1$, we can doubtless safely say that the difference is *significant*. It will be convenient to use this term in the following paragraph.

We first compare the A. M. value during relaxation with that of the preceding period where the subject was requested not to relax: The jerk decreases during the first period of relaxation in all but one of 36 instances; it decreases during the second period of relaxation in 12 out of 12 instances; likewise it decreases during the one instance we have of a third period of relaxation. In sum, a significant decrease of jerk is found in 48 out of 49 instances of relaxation as compared with the preceding period where the subject is requested not to relax.

We now compare the A. M. value *after* each period of relaxation with that of the preceding period of relaxation: In the first after-period an increase occurs in 19 out of 36 instances, but only 12 of these are significant, while in the second after-period an increase occurs in 9 out of 12 instances, 8 of which are found to be significant. Accordingly the A. M. jerk after relaxation significantly exceeds that during relaxation in 20 out of 48 instances.

Study of the individual records of the foregoing 2 series reveals the following items of interest: 1. In the case of J. C., where, contrary to all other instances, the jerk increases during the first period of relaxation as compared with the preceding period, marked discomfort and consequent failure to relax owing to distress from unusually tight strapping was reported. However, during the second period of relaxation, the A. M. jerk is less than that of the preceding or succeeding periods where the subject

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Fig. 1. The unbroken line indicates decline of the knee-jerk averaged for 13 medical students during 25 minute period reading aloud or writing. Ordinates indicate extent of jerk in centimeters. Stimuli at 30 second intervals. This curve has been smoothed, but the original points are shown.

The broken line is the curve of relaxation for the same students. This has been plotted from the reciprocals of the values (ordinates) in the foregoing curve multiplied by 10; that is, the ordinates in the broken line represent reciprocals of extent of jerk (in centimeters) $\times$ 10.

Fig. 2. Subject J. K., untrained neurotic, reading aloud, 7–21–1927. Stimuli at 30 second intervals. Failure is noted of knee-jerk to diminish, probably indicating absence of relaxation (see text). Space has been conserved in printing this graph by omitting the portion below 5 cm., because no jerk fell below this value.

Fig. 3. Subject H., experienced at relaxation, reading aloud, 12–4–1926. Stimuli at 30 second intervals with pauses as indicated on abscissas. Graph shows diminution of knee-jerk (often to zero value) during relaxation.

Fig. 4. Subject I. B., experienced at relaxation reading aloud, 11–21–1926. Conditions as in figure 3. Graph likewise shows diminution of knee-jerk during relaxation.

Fig. 5. Subject same as in figure 4, writing numbers, 11–28–1927, but requested *not* to relax. Graph indicates failure of knee-jerk to diminish.

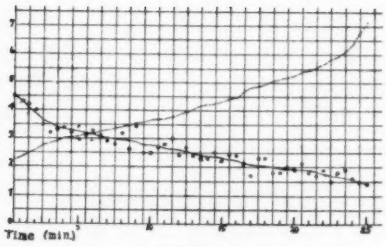

Fig. 1

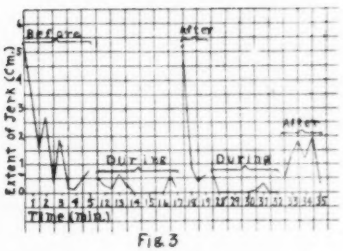

Fig. 3

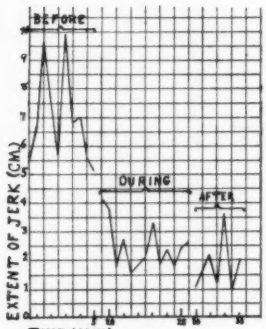

Fig. 4

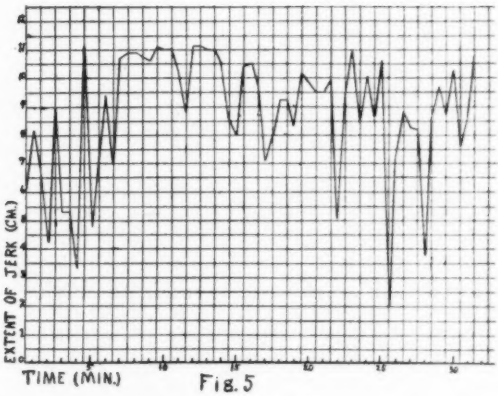

Fig. 5

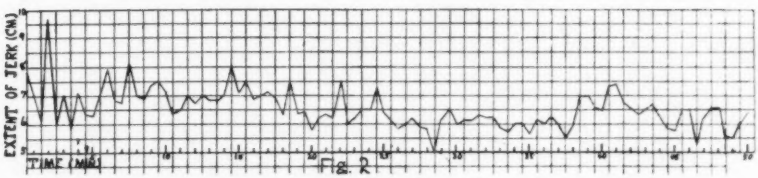

Fig. 2

TABLE 1

Arithmetic mean values of knee-jerk of trained subjects during relaxation compared with values before and after relaxation

Abbreviations: Occ. = occupation of subject during tests; r = reading; w = writing copy from a magazine; m = writing numbers regularly to beats of a metronome; A.M. = Arithmetic Mean value; σ = Standard Deviation; P.E. = Probable Error; R = ratio of the difference between the adjacent A.M. values to the sum of the corresponding probable errors. Where $R \lesseqgtr 1$, it is presumed to be significant. So calculated, the A.M. jerk during relaxation is diminished as compared with the foregoing period in about 98 per cent of the instances; while after relaxation the jerk increases over that during relaxation in about 42 per cent of the instances.

NAME	DATE	OCC.	BEFORE			R	DURING			R	AFTER			R	DURING			R	AFTER		
			A.M.	σ	P.E.		A.M.	σ	P.E.		A.M.	σ	P.E.		A.M.	σ	P.E.		A.M.	σ	P.E
I. B.	November 21, 1926	r	3.2	1.5	2.8	7.1	0.32	0.45	0.08	0.13	0.34	0.40	0.07								
H. G.	November 16, 1926	r	3.69	0.37	0.09	7.4	1.75	0.94	0.16	0.48	1.63	0.37	0.09								
H. G.	November 20, 1926	r	6.43	0.61	0.09	2.05	3.76	0.14	0.04	0.22	3.78	0.22	0.05								
L. G.	November 29, 1926	r	0.16	0.10	0.02	8.0	0	0	0	6.0	0.06	0.04	0.01								
G. H.	December 4, 1926	r	1.72	1.54	0.32	4.14	0.23	0.22	0.04	2.2	1.77	1.98	0.66	2.21	0.22	0.24	0.04	6.4	1.18	0.59	0.11
G. H.	December 11, 1926	w	1.33	0.92	0.19	6.04	0.36	0.39	0.09	0.63	0.55	0.88	0.21	0	0.55	0.58	0.14	1.16	0.26	0.52	0.11
M. F.	November 29, 1926	r	2.25	1.50	0.26	4.84	0.75	0.37	0.50	3.40	1.70	0.92	0.23								
M. F.	December 13, 1926	w	5.25	1.62	0.31	4.35	2.51	1.54	0.32	0.72	2.12	1.24	0.22								
M. F.	December 7, 1926	r	2.32	1.94	0.43	2.67	0.53	1.02	0.24	1.82	1.44	0.97	0.26	0.77	1.06	0.99	0.23	0.58	0.88	0.31	0.08
C. B.	November 17, 1926	r	0.85	0.24	0.06	2.83	0.51	0.31	0.06	1.28	0.74	0.51	0.12								
E. J.	November 13, 1926	r	2.77	0.62	0.17	7.08	1.07	0.43	0.07	0.71	1.27	0.93	0.21								
E. J.	November 14, 1926	r	2.32	0.75	0.20	6.14	0.53	0.35	0.09	1.69	0.31	0.14	0.04								
E. J.	November 14, 1926	r	3.51	0.88	0.22	7.23	1.05	0.77	0.12	1.9	0.67	0.30	0.08								
E. J.	November 16, 1926	r	2.90	1.34	0.37	4.0	1.18	0.30	0.06	0.23	1.21	0.33	0.07								
E. J.	November 19, 1926	r	1.58	1.03	0.24	2.96	0.75	0.40	0.04	3.62	0.46	0.14	0.04								
E. J.	November 26, 1926	r	1.24	0.28	0.07	5.06	0.43	0.51	0.09	0.12	0.41	0.31	0.07								
H. K.	November 20, 1926	r	2.56	0.38	0.03	1.53	1.49	0.41	0.04	0.13	1.43	0.41	0.04								
H. K.	November 27, 1926	r	7.93	1.21	0.38	5.05	5.45	0.96	0.21	3.17	4.12	0.94	0.21								
D. M.	December 3, 1926	r	0.93	0.75	0.13	5.18	0.05	0.19	0.04	1.25	0	0	0								
D. M.	December 8, 1926	w	5.64	1.52	0.32	14.63	0.52	0.14	0.03	2.52	1.20	1.16	0.24	3.7	0.20	0.10	0.02	2.16	0.33	0.20	0.04

E. R.	July 16, 1926	r	3.32	0.24	0.07	8.64	2.11	0.33	0.07	0.5	2.04	0.24	0.07							
S. W.	December 8, 1926	w	10.84	1.07	0.22	9.19	7.90	0.48	0.10	5.3	8.33	0.81	0.17	3.61	7.50	0.28	0.06	5.92	8.27	0.31	0.07
S. W.	December 15, 1926	w	13.55	0.46	0.13	11.0	10.58	0.50	0.14	1.08	10.32	0.36	0.10								
B. R.	November 30, 1926	r	3.84	1.65	0.37	3.33	1.91	0.96	0.21	1.5	1.34	0.73	0.17								
B. R.	December 27, 1926	w	5.17	1.82	0.38	5.20	2.36	0.79	0.16	1.93	2.96	0.74	0.15	0.56	2.78	0.81	0.17	0.07	2.81	1.19	0.25
B. R.	December 13, 1926	w	5.26	1.42	0.30	2.20	4.07	0.99	0.24	0.7	3.69	1.49	0.30	2.15	2.66	0.87	0.18	1.02	2.21	1.20	0.36
B. R.	December 7, 1926	r	2.09	1.43	0.26	2.05	1.25	0.86	0.15	6.07	3.68	0.75	0.25	2.46	2.72	0.63	0.14	1.82	4.36	1.01	0.27
B. R.	January 14, 1927	w	3.80	1.05	0.22	1.07	2.69	1.22	0.25	0.58	2.44	0.93	0.20								
M. F.	January 3, 1927	w	3.04	0.87	0.19	2.19	2.23	0.85	0.18	0.31	2.32	0.52	0.11	2.5	1.82	0.42	0.09	1.67	2.19	0.62	0.13
H. S.	January 15, 1927	w	1.15	0.65	0.14	8.21	0	0	0		0	0	0								
J. C.	January 15, 1927	w	0.90	0.73	0.15	1.10	1.33	1.15	0.24	3.7	3.29	1.36	0.29	1.21	2.78	0.63	0.13	5.55	5.40	1.08	0.26
B. R.	January 23, 1928	m	2.75	1.58	0.33	2.66	1.39	0.89	0.18	5.04	2.80	0.45	0.10	10.86	0.52	0.52	0.11	1.0	0.74	0.52	0.11
E. J.	August 5, 1927	m	2.92	1.73	0.38	5.29	0.38	0.54	0.10	2.72	1.63	1.76	0.36	1.65	0.88	0.50	0.10				
I. B.	November 1, 1927	m	3.23	1.50	0.28	8.08	0.32	0.45	0.08	0.13	0.34	0.40	0.07								
B. R.	October 30, 1927	m	1.23	2.14	0.31	1.58	0.52	0.77	0.14	2.56	0.11	0.14	0.02								
M. F.	November 2, 1927	m	3.96	1.77	0.32	1.88	2.89	1.91	0.25	1.87	2.00	1.15	0.22	1.19	1.39	1.45	0.30	1.59	3.00	3.17	0.71
						1.23	1.88	0.99	0.20												

was requested not to relax. We can readily understand that the individual may fail at times to relax in the presence of distress. Our aim was to avoid drawing the straps so tight as to cause distress, because we did not wish to have this obstacle to relaxation; but in this one instance, we failed. 2. A subjective experience of relaxation (i.e., the absence of sensations from muscular contraction) was often reported by the subjects, agreeing in time with the period of diminished jerks. More precise tests on this point are planned. 3. Comparing records of the knee-jerk secured in different months without any voluntary attempt to relax, there are indications that as relaxation is practiced and becomes habitual during the course of months, the knee-jerk tends to undergo a gradual diminution and becomes more uniform. The records of subject M. F., epileptic, before training were marked by extreme inequality in the extent of jerks, low and very high ones occurring irregularly; that this was due to her emotional state seemed probable from her subjective reports, her occasional sighs and the frequently contracting and relaxing muscles of her forehead and brow. A year later, when she no longer appeared or reported being emotionally disturbed, her tracings show greater uniformity and some diminution. The jerk of another subject, L. G., although not high in 1925, had become so low in 1927 that it could scarcely be elicited without augmentation. D. M. appeared to give a lower jerk in 1927 than in 1925, and in E. J. very marked diminution was noted in 1927. However, the characteristics of knee-jerk curves of neurotics, we find, vary considerably from day to day. Accordingly, these observations are incomplete and too few in number to permit more than a tentative conclusion. 4. Three subjects (H. S., partially trained, E. F. and E. J., well trained) ordinarily gave low jerks. One (E. F.) expressed doubt in advance that she would be able to carry out the instruction to read without relaxing, for she stated that for more than 6 months previously she had practiced at relaxing while reading and she believed that the combination had become habitual. Another (H. S.) volunteered that for years she had habitually relaxed while reading but also had "gone to sleep that way each night" and she too believed that she had formed an association. To the third (E. J.) it seemed evident that as a rule his leg-muscles were highly relaxed. Assuming that these individuals are correct in their autosensory observations, we can readily understand their low jerks as due to the habitual relaxation they report. However, it would be hasty and unwarranted to assume that a low knee-jerk is a necessary and sufficient mark of an habitually relaxed individual, always indicating the absence of nervous hypertensive symptoms. For in the writer's clinical experience, although high jerks predominate in nervous hypertensive conditions, low jerks are not infrequent, even in the absence of all organic nervous derangement.

Series 5 was performed with 5 trained subjects for purposes of control.

They were requested not to relax throughout the period, an instruction which was occasionally repeated. Nevertheless all reported a tendency to relax at times. One (M. F.) tried to correct this by special attention to the occupation. Another (D. M.) was observed to yawn. The occupation was writing numbers to the beats of the metronome, except with one subject (M. F.) who reported that she experienced a relaxing tendency from the reported beats and preferred to write copy from a magazine. In the cases where the metronome was used, the rate was set well below the speed of each individual, except in one (E. J.). Here we secured almost no jerk with a low rate from the outset and in order to produce a jerk it was necessary to set the metronome rate well above his speed. Even then, the subject reported a very strong tendency to relax. The subjects did not seek to overcome this tendency by voluntary stiffening of muscles. Likewise the general conditions maintained were the same as those in the foregoing 4 series: we sought to avoid noises, conversation and disturbances on the part of the experimentor that might stimulate or excite the subject. He was in each case instructed not to relax, but if nevertheless he tended to relax, he was permitted to do so. If we except the one record (E. J.) where failure to keep from relaxing was reported, the records are in obvious contrast with those of series 3: Where the subject was requested not to relax and reported relative success in following the instruction (4 instances out of 5), the knee-jerk was not markedly decreased (fig. 5). These records of trained subjects requested not to relax bear comparison with those of untrained neurotic subjects who in *series 2* failed to relax: they are alike in that the apparent absence of relaxation was attended by failure of the knee-jerk to show the diminution characteristic of periods of relaxation. The results of series 4, 5 and 6 therefore tend to confirm our conclusions from series 1 and 2 that differential relaxation produces a diminished jerk.

Graphic tests of position of knee. In about half of our records with trained subjects and in most of those with untrained subjects (excluding the neurotic and under quiet conditions) a general decline in the magnitude of jerk had been observed during the course of the period. This aroused our suspicion that unknown to the subject slight shifting of the knee might have occurred, diminishing the knee-jerk when a less sensitive area of the patellar tendon was stimulated. We sought to minimize the effects of any possible forward or backward shifting of the knee by making the unimpeded stroke of the hammer relatively long, namely, 66 mm. Apparatus to record movement of the knee, either forward or lateral or lateral rotation, is described above under "Methods." With 4 trained and 3 untrained subjects, records of knee-jerks were taken on one kymograph, while the position of the knee was recorded on another.

We wish to test whether the knee-jerk can diminish during a period of

relaxation with a trained subject, then increase in height after relaxation, while all the while the resting position of the knee remains practically constant. That such diminution can occur in the absence of forward shifting was clearly shown by the tests with both classes of subjects. In all instances no shifting occurred laterally or as lateral rotation, and in all instances with several exceptions the forward deviation of the knee was zero or approximately less than 1.5 mm., which evidently was negligible in comparison with 66 mm. of possible thru t of the hammer. In the exceptional cases, where in spite of all strapping, the resting-position of the knee shifted forward or back a short distance such as 2.5 mm., examination of the record disclosed the absence of correlation between decline of the jerk and change of position of the knee; that is, if the knee deviated 2.5 mm. forward for a time, the jerks during this time were not characteristically diminished but were often equal or in some instances increased. Accordingly we secured a double line of evidence that the diminution of the jerk during our periods of relaxation could not be attributed to shifts of the knee.

Results with reinforcement. As has been well known since the time of Jendrassik (1883) and other early writers (Mitchell and Lewis, 1886; Lombard, 1887), vigorous voluntary movements tend, if appropriately timed (Bowditch and Warren, 1890), to increase the knee-jerk. Their evidence makes it seem safe to say that the marked contraction of almost any skeletal muscle group tends to have this effect. But the present investigation discloses that this does not always hold true for the extremely relaxed state. After extreme relaxation has once been induced, not only does it tend to hold over in the subsequent period as we previously found (loc. cit.), but moderate contraction of almost any muscle group (not of the leg where tracings are being taken) may fail to restore the jerk to the extent it had before relaxation. Even Jendrassik's method of pulling apart the two hands with fingers interlocked, when moderately performed, occasionally fails to produce a high kick, although this effect generally occurs if the pulling is vigorously repeated. This method of Jendrassik was used in 9 untrained subjects of series 1. In the majority of these (5) such reinforcement failed to restore the jerk to the original height; in 2 instances it was restored to approximate equality; in 2 instances it became greater than the mean of that set. One subject who was tested on two occasions in this manner showed a difference of effect of such reinforcement, for on one occasion the jerk considerably failed to be restored to its previous extent while in the other the opposite condition took place. Likewise other types of reinforcement (clenching the left fist, rotating the left hand, several minutes of general muscular exercise, wrinkling the forehead, deep breathing, conversing in addition to writing) were used, but these as a rule failed to restore the jerk to the original, being less effective than the Jendrassik

type of reinforcement; except when reinforcement took the form of voluntary stiffening of the legs or thigh, particularly the right, which seemed a very effective form. In series 3 and 4 with trained subjects, Jendrassik reinforcement, which was performed toward the end of the period in 19 instances, restored the jerk to more than the extent present before relaxation in 13 instances, to about equal in 3 instances, while in 3 others the reinforced jerk was considerably less than the original extent. The greater restorative effect of such reinforcement in series 1 as compared with series 3 and 4 is perhaps to be explained because in the latter, reinforcement took place not during the relaxed period but at a time when the subject had been directed to cease relaxing. However, since we can not be certain that this is the correct explanation, the experiment should be repeated with reinforcement occurring during the relaxing period with trained subjects, and the conditions should be made precise so that the muscular work performed in the act of reinforcement could be calculated in ergs.

In harmony with the above findings on the effects of reinforcement during relaxation are some observations of Bodwitch and Warren (1890) under conditions where their subjects became drowsy. During this state the jerk of one subject, which without reinforcement normally averaged 83 mm., decreased during 18 tests to an average of 17 mm. The reinforced jerk during the drowsy state as compared with the wide-awake state was greater than the non-reinforced jerk, but 60 to 100 per cent smaller than the reinforced jerk.

Discussion. That the diminution of the knee-jerk in trained and in untrained subjects under our conditions was due to muscular relaxation and not to other factors is evidenced by the following considerations:

1. As has previously been shown, the knee-jerk tends to diminish with advanced general relaxation, furnishing us with a possible test for relaxation.

2. Subjects experienced at relaxation reported a diminished sense of tenseness during the periods devoted to relaxation, when the corresponding tracing revealed a diminished knee-jerk. Certain of the untrained subjects likewise spontaneously reported during such periods that they had felt relaxed or drowsy or "had nearly gone to sleep."

3. Both the trained and untrained subjects seemed to the investigator to manifest external signs of relaxation, as shown by a less alert manner, more drowsy appearance, lessened contraction of visible muscles of the forehead, brow, and face and lessened stiffness of posture. In some instances there was a manifest appearance of "not working so hard" as they wrote or a certain lassitude of voice when reading aloud during differentially relaxed moments.

4. When trained subjects were requested not to relax, and apparently

did not relax, the knee-jerk did not show marked diminution, although other conditions were maintained as in previous series where diminution of the jerk had occurred.

5. Diminution of the jerk could be prevented by whatever apparently prevented relaxation, as exciting occupation, increasing the metronome rate so that the subject wrote more rapidly, disturbance by the investigator, and muscular reinforcement.

6. Three neurotic subjects, who evidently did not relax, correspondingly did not show a diminished jerk.

7. The observed diminution of the knee-jerk evidently was not due to slipping of the knee or slipping of the hammer from its original position as shown by various tests:

a. Tests made with levers as above described disclosed no movement or practically no movement under our conditions with the thigh strapped in place.

b. When the jerk had become diminished apparently through relaxation, change of position of the hammer failed to restore the jerk to its initial height. This was true for both trained and untrained subjects. In some experiments the hammer was thus adjusted from 4 to 10 times in vain.

c. The hammer was prevented from slipping by clamps and adjustments of a particularly firm nature. Movable joints, where not absolutely required, were soldered.

8. Fatigue was not the source or at any rate not the principal source of the observed diminution of the jerk:

a. The subjects did not as a rule report fatigue either generally or at the site of stimulation.

b. General fatigue probably did not occur since reading, which is a lighter occupation than writing, surely could not as a rule cause such a condition within 15 to 25 minutes. Furthermore, no evidence is found in past investigations which would lead us to expect fatigue arising in consequence of brief periods of mental work to result in diminution of the jerk. We owe to Lombard (1887) our current conception that fatigue can have such a result, but he emphasized that he meant "the amount of fatigue such as would ordinarily occur during the course of the day" (p. 48). He is careful to say, "In our experiments we have not found that short periods of mental work have any effect on the knee-jerk" (p. 45).

c. Diminution of the knee-jerk could be made to occur relatively early in the curve if the request to relax was given at such a time,—evidently too early for fatigue to have set in as a rule, either general or local.

d. Fatigue of the sensory end-organ (muscular, according to Sherrington, 1915) certainly was not generally the cause of the observed diminution of jerk in untrained subjects, since in those instances where an interval of 5 to 15 minutes took place free from stimulation by the hammer, the drop

in the height of jerk following this interval was fully as marked as when the hammer continued to strike without interruption.

e. Likewise, fatigue at the site of stimulation evidently was not the responsible factor since, when the jerk was diminished during relaxation, changes of the position of the hammer failed to restore the jerk, while

f. Reinforcement due to voluntary movements of the subject or the occurrence of noises (exceptions noted above) brought back a strong jerk.

g. In trained subjects who were requested not to relax and who reported success (series 5), diminution of the jerk did not set in or was not equal to the above.

h. In dogs, after a preliminary diminution, Johnson and Luckhardt (personal communication) have secured knee-jerk curves for periods as long as 17 hours without marked further diminution. Evidently the dog's end-organs do not readily fatigue. While this result may not hold equally for man, it seems unlikely from this that end-organ fatigue in man should set in within a fraction of an hour.

i. With trained subjects, as recounted above, there were 21 out of 49 instances where subsequent to relaxation the jerk increased. This would not be expected if fatigue, rather than relaxation, were to be regarded as the cause of the diminution of the knee-jerk.

9. Interference with circulation or other effects of constraint due to strapping were not responsible factors; for when the jerks had become diminished, they remained so if the straps were removed, and if the straps were not used at all during the entire period, the diminution of the jerk occurred nevertheless.

Luckhardt and Johnson (personal communication) have observed in spinal animals a fall in the jerk owing to causes as yet unanalyzed. It is likely that such falls can be produced by causes other than relaxation, such as 1, diminishing vitality or approaching death of the animal; 2, local injury from blows of the hammer; 3, shifts of position of the hammer or of the patellar tendon; 4, fatigue at some stage; 5, the action of anesthetics or other toxins, and 6, possibly other unknown causes. Since manifestly there are numerous possible causes of fall of the knee-jerk, we can not under any particular set of conditions state the cause unless there is evidence that enables us to discriminate. Under the present conditions with human subjects, evidence was given above that the responsible factor was relaxation.

Lombard recorded his own jerks 2321 times during 14 successive days. Although he does not mention the fact, his figures show a marked diminution of the average height of jerk per day as the days pass. Thus the average height on the first day was 63 mm., while on the 14th day, 35 mm. It is difficult to account for this decrease except on the basis of progressive differential relaxation.

It is of interest to offer a definition of the type of relaxation which has here been investigated. If by *general* relaxation we mean the relaxation of practically all of the skeletal musculature when lying down (excepting several groups, including the sphincters, the muscles that close the jaw, the *orbicularis oculi* when the eyes are closed), then the relaxation under the conditions of present investigation can be readily described. *Differential relaxation apparently means the absence of an undue degree of contraction in the muscles employed for an act or for posture, while other muscles, not so required, are flaccid.* We have found that differential relaxation, as tested by the knee-jerk, is produced in trained subjects, and exists under favorable conditions in the untrained normal individual. Our observations with untrained neurotic subjects indicate that some of these fail to give what we have regarded as a normal curve of relaxation under our conditions, suggesting that this is a possible test for nervous hypertension or neurosis. We know, however, that sometimes the neurotic individual succeeds in relaxing during the period, giving a normal curve, and that therefore the test while promising to be useful, is not infallible, but must be considered along with other data.

CONCLUSIONS

1. In experiments on 21 subjects without preliminary training to relax it was found that continuous reading or writing numbers or copying generally permitted sufficient relaxation of the mechanism of the jerk to register marked decrease in its amplitude during the course of the period.

2. Decrease of the jerk tended to be prevented by whatever seemed to have a stimulating effect, including exciting conversation, noises, disturbance by the investigator and vigorous voluntary movement.

3. Decrease of the jerk even under favorable conditions fails to occur in some untreated neurotics.

4. Fourteen subjects trained to relax engaged in the same occupations during a control period, a subsequent period of relaxation and a period after relaxation. During relaxation the jerk almost invariably diminished as compared with the preceding period, and was frequently less (42 per cent of instances) than during the subsequent period.

5. The effects of relaxation generally tended to hold over into the after-period.

6. It is generally possible to prevent the diminution of the knee-jerk in trained subjects during a prolonged test by instructions not to relax.

7. Evidence is presented that the above decrease in the knee-jerk is due to differential relaxation.

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STUDIES ON THE OESTROUS CYCLE IN THE RAT

IV. The Basal Metabolism during the Oestrous Cycle[1]

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The development of a simple method of following closely the oestrous cycle in the rat, and the possibility of correlating this with the menstrual cycle of the primates, makes it profitable to determine carefully the changes in the basal metabolic level during the oestrous cycle. This is especially true in view of the considerable amount of contradictory evidence that has been published on the effect of the menstrual cycle on basal metabolism.

A number of investigations have been reported which indicate that menstruation has no appreciable effect on the basal metabolic rate. Zuntz (1906) found that in two women observed daily for three months there was no correlation of the variations in carbon dioxide production with the inter-, pre- and post-menstrual phases of the cycle. Blunt and Dye (1921) in a series of 216 observations in 17 women found no definite changes in the basal metabolism previous to, during, or after menstruation. The published data of Blunt and Dye show, however, that the average metabolic rate of their subjects was somewhat increased on the first day of the menses or the first day before, and Wakeham (1923), who has also analyzed their data, concludes that a rise in the late premenstrual and a fall in the menstrual phases did occur. Wiltshire (1921), on the basis of observations on five subjects each day during the menstrual periods and three or four times between periods, concluded that the results could not be regarded as showing any marked effect due to menstruation. She also found no difference in the cost of doing work at the different periods. The published data of the determinations in the five subjects show, as in the subjects of Blunt and Dye, that the highest rate of metabolism occurred in the premenstrual phase, the lowest in the postmenstrual and an intermediate rate in the intermenstrual. The data are too few, however, to make valid any conclusions from the statistical analysis. Boothby and Sandiford (1924) reported that they found no constant variation of basal metabolism with the menstrual cycle, and suggested that the increases

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reported by others have been due to pain and discomfort in the subjects during menstruation. This explanation can hardly apply, since the increase in the metabolic rate has been reported as occurring not only in the early menstrual, but also in the late premenstrual phase, before the flow has commenced and probably before pain or discomfort is felt. Also, all investigators who have found definite effects have reported a decrease in the basal metabolic rate soon after the establishment of the catamenia, and during the time when discomfort or pain might be present.

On the other hand, a number of investigators have reported definite changes in the basal metabolic rate during the menstrual cycle. Snell, Ford and Rowntree (1920) found that in eight out of ten cases an increase of from 4 to 14 per cent in the basal heat production occurred early during menstruation or in the premenstrual period, and was followed by a postmenstrual fall. Rowe and Eakin (1921) reported that in their series of six subjects they found a uniform rise occurring within the week preceding the onset of the menses, and a fall during the menstrual period and the two weeks following. Wakeham (1923) made 98 determinations on 24 subjects who were living closely ordered lives under very uniform conditions. His carefully analyzed results show a premenstrual increase in metabolism beginning less than a week preceding menstruation, followed by a decrease below normal at the beginning of, or during the menstrual period. A gradual return to the normal followed during the next 7 to 10 days. This finding was made only by treating the results statistically, since the daily fluctuation due to other factors at first obscured the change due to menstruation.

Examination of these investigations indicates clearly that no very marked change in the basal metabolic rate during the cycle of changes in the ovary is to be expected. That the internal secretions of the ovary have a much less effect on the basal metabolic rate than those of the thyroid or pituitary is indicated by the fact that spaying in animals decreases the metabolism only slightly, and that metabolism after the menopause in women is, comparatively, only slightly changed. The suggestion is persistent, however, that there is some change in the metabolic level with the ovarian cycle, and that this change falls within the plus or minus 10 per cent variation in metabolic rate which is ordinarily allowed as experimental error. The reasons for the discrepancies in the investigations reported would seem to be: a failure to recognize that any change in the metabolic level must necessarily be small; the notion that any changes in metabolic rate should come at the time of menstruation; the failure to make a large number of determinations in a sufficient number of subjects, during all stages of the cycle, so that the data might be treated statistically; the impossibility of following the cycles except by the occurrence of the menses;

and finally the probability that menstruation might have occurred without ovulation.

In all of the investigations hitherto reported the chief emphasis has been placed upon the period of menstruation itself, although it is now generally agreed that this is but a superficial manifestation of the cycle of ovarian changes. There are two other events in the cycle which are of far greater physiological moment when they are manifested, but which may be omitted from a menstrul cycle entirely, and this without giving any outward indication of their omission. These events are the growth, maturation and rupture of a follicle, and the development, growth and degeneration of a corpus luteum. The recent work of Corner (1923) and Allen (1927), together with many older reports, and observations in surgical cases, indicates that menstruation may occur without ovulation, and hence without corpus luteum formation. Both Corner and Allen found in their monkeys at autopsy that this condition had occurred repeatedly in more than half. The menstrual period that occurs without a preceding ovulation, and hence without a corpus luteum degeneration, is outwardly the same as any other catamenia, but is profoundly different in the character of the endometrial changes, as Corner (1927) has shown. Although the cause of menstruation itself is not known as yet, its occurrence without a preceding ovulation and corpus luteum formation might be expected to have little or no effect upon basal metabolism.

Some of these objections may be met by the use of experimental animals which can be kept under very constant environmental conditions, which can be used for determinations at any time, and upon which a large number of observations can be made. The rat is an excellent animal for such purposes. By means of the vaginal smear method, developed for the rat by Long and Evans (1922), it is possible to follow the course of the oestrual cycle with a considerable degree of accuracy. If smears are taken at intervals of about three hours, the beginning and end of each stage may easily be determined to within an hour. Ovulation is known to occur in stage III (Long and Evans), or approximately 36 hours after the beginning of stage I. The cycle in the rat, although it is homologous with the menstrual cycle in the primates, is still quite different from it. There is nothing in the oestrous cycle representing menstruation, and unlike the menstrual cycle, follicle and corpus luteum formation are probably necessary concomitants.

Methods and results. Thirty-six female rats, from four to seven months of age and in good health, were selected from the colony for the series. Oestrual cycle determinations by the vaginal smear method were made on these rats for a preliminary period of about thirty days, in order to have an exact oestrus history on each animal. Of the 36 animals, 14 which showed regular cycles of from four to five days duration were finally

selected for the metabolism tests. All of the other 22 animals of the original series showed more irregular cycles than did the 14 selected, although the total average length of cycle for the whole group in the preliminary period was 4.8 days, a figure which is considered normal. Of the 14 rats selected for the metabolism series, only nine were used for the data of this paper. One rat was killed accidentally and four showed, during the course of the metabolism experiments, oestrual cycles sufficiently abnormal to warrant discarding them. The nine animals that were used

TABLE 1

Oestrual histories of rats used in metabolism determinations

RAT NUMBER	AGE AT BEGINNING OF EXPERIMENT	WEIGHT AT BEGINNING OF EXPERIMENT	WEIGHT AT END OF EXPERIMENT	LENGTHS OF SUCCESSIVE CYCLES			
				A. Preliminary period	Average (A)	B. During period metabolism determination	Average (B)
	days	*grams*	*grams*	*days*	*days*	*days*	*days*
4370	172	152	178	5, 4, 4, 5, 4, 6, 4,	4.6	5, 4, 4, 6, 4, 4, 5, 4, 4, 5, 5, 4, 4, 4, 4	4.4
1265	201	186	230	4, 4, 6, 4, 4, 5	4.5	4, 4, 6, 5, 5, 4, 5, 4, 5, 4, 4, 5, 4, 5	4.6
4355	197	178	206	3, 4, 6, 5, 4, 5	4.5	6, 5, 5, 4, 4, 4, 4, 5, 5, 4, 4, 5, 5	4.6
518	186	161	178	4, 5, 5, 5, 4, 6	4.8	4, 4, 7, 4, 5, 4, 4, 5, 5, 4, 4, 5, 4	4.5
2660	194	180	228	6, 4, 5, 4, 5, 5	4.8	3, 4, 4, 5, 4, 4, 4, 4, 5, 4, 5, 4, 4, 5, 4, 4	4.2
4350	187	164	186	5, 5, 4, 5, 4, 5	4.7	4, 5, 5, 4, 4, 4, 5, 4, 4, 4, 5, 6, 4, 4	4.4
4371	172	150	192	5, 4, 4, 6, 6, 4	4.8	4, 6, 4, 5, 4, 4, 5, 4, 4, 4, 4, 7, 4, 5	4.6
4335	189	167	188	4, 4, 5, 6, 5, 4	4.7	5, 4, 6, 5, 5, 6, 4, 4, 4, 5, 4, 4, 4, 5	4.7
2661	194	174	226	4, 4, 4, 5, 4, 5	4.3	5, 5, 4, 4, 4, 5, 4, 4, 5, 4, 4, 4, 5, 4	4.4
	Averages				4.6		4.5

showed cycles of rather uniform length, averaging 4.6 days in the preliminary period and 4.5 days in the experimental period. The complete oestrual histories of these rats are given in table 1. These animals were thus several times selected, first as being healthy animals of sleek coat and normal weight, then selected as having normal oestrous cycles and being able to maintain these cycles during the whole period.

All of the animals maintained or gained body weight throughout the experiment. The regimen of some of these rats was rather severe, in-

volving periods of starvation of from 18 to 24 hours followed by a period with food of only 24 hours, or, in some cases, of only three hours. In order to stimulate the animals to eat heartily when they were returned to the feeding cage after being starved, a variety of food was offered them, green lettuce or cabbage, corn, wheat, milk and small amounts of cooked meats besides the usual biscuit. The fact that the rats were able to exhibit regular and rather uniform oestrous cycles during the experimental period and also to maintain or to gain weight, even though subjected to the often repeated starvation periods indicates that there was no condition of inanition. This is considered important in view of the fact that the functioning of the ovary appears to be a sensitive indicator of the general well-being of the animal. All of the animals at the end of the experiment appeared to be in excellent health and three which were killed and autopsied showed no signs of disease. All of the animals were thoroughly gentled.

It is manifestly impossible to make basal metabolic rate determinations at intervals of an hour or so throughout the course of a single cycle in an individual rat. A starvation period of 18 hours must precede each series of tests. Furthermore, it is not advisable to starve the rats longer than 24 hours, since the respiratory quotient changes with starvation, and since the chance of error in a method based on carbon dioxide determination is comparatively great if the respiratory quotient changes. The course finally adopted was briefly as follows. A rat was starved for approximately 17 hours and then a series of determinations was made for six hours. Oestual cycle observations were made at intervals of three to ten hours during the starvation period, and at the end of each second or third metabolism test. The rat was then returned to the feeding cage for a period of three to twenty-four hours and again starved for 17 hours. This procedure was repeated until several cycles had been passed. Occasionally a rest period of several days was given to the rat. From the oestrus history it was possible to predict the time of occurrence of particular phases of the cycle in which metabolism determinations were desired. This was often done, especially for the portion of the cycle in which marked variations were found.

A modification of the gravimetric method of Haldane was used in the metabolic rate determinations. The rat was confined in a small airtight chamber in the middle of a train of tubes containing absorbents and through which warm air was drawn by means of a water vacuum pump. The rate of ventilation was kept at about 700 cc. per minute. The metabolism train was composed of the following units: 1, a Vanier drying bulb containing sulphuric acid, serving to dry the incoming air and also to indicate by the rate of bubbling, the rate of passage of the air through the system; 2, a Blount gas absorption tube containing an as-

bestos-sodium hydroxide preparation for CO_2 absorption; 3, a Blount tube containing magnesium perchlorate trihydrate for water vapor absorption (Lee and Brown, 1927); 4, the animal chamber; 5, two Blount tubes containing magnesium perchlorate trihydrate for water absorption; 6, two Blount tubes containing each in the one side asbestos-sodium hydroxide for CO_2 absorption and in the other side the water absorbent. The gain in weight of each tube was determined to 0.1 mgm. In part of the work a tube containing clean pumice stone saturated with water was placed just before the animal chamber. This precaution was subsequently found to be unnecessary. The rats were apparently as comfortable in the dry as in the moist air. The Blount tubes are especially suitable for such absorption work because of their lightness and the fact that the water liberated on the one side by the reaction of carbon dioxide with sodium hydroxide is absorbed on the other side of the same tube and weighed at the same time. In the greater part of the work two trains, separate except for the common animal chamber, were used. In the ordinary routine, a metabolism test of about half an hour was made with one train, and then the air switched into the other train for a similar period for a check determination. While this was in progress the tubes of the first train were weighed. All determinations were made over periods of 25 to 35 minutes, timed with a stopwatch. The CO_2 production was measured by the gain in weight of the last two Blount tubes. The oxygen consumption was measured by the total gain in weight of the whole system during the experiment, since oxygen was the only thing that was added. From the carbon dioxide production and the respiratory quotient, the heat production per day was calculated.

The animal chamber was a pint glass jar, large in diameter (10 cm.) and short in height (10 cm.). This type of jar accommodates a rat weighing as much as 300 grams without apparent discomfort. The bell shape encourages the animal to keep to the bottom and allows it to lie curled naturally on its side. That the chamber is comfortable is shown by the fact that if such jars are placed in a large cage the rats will enter them voluntarily, curl up and go to sleep in them. The chamber was kept at a temperature of 29 to 30°C. by means of a carbon filament bulb placed at a suitable distance. This temperature is slightly above the critical temperature (Goto, 1923), and under such conditions the rats usually become quiet quickly and spend much of the time dozing. The light shining on the jar also served somewhat to prevent bodily movements. Previous to a determination each rat was given a rest period of about an hour in the cage with the air circulating through the waste train. During the test, if any movements greater than raising the head occurred, the determination was stopped and at least five minutes were allowed to elapse after the movements ceased before continuing. It was not at all uncommon for

these rats, accustomed as they were to the chamber, to remain without a perceptible body movement for periods as long as thirty minutes. Any test in which the animal showed persistent restlessness was discarded, even though the actual determination was made only during periods of rest.

Body surface was calculated from the Meeh formula

$$S = k\,W^{\frac{2}{3}}$$

in which S is the area in square centimeters, W is the weight in grams and k is the constant, 11.36, determined by Carman and Mitchell (1926).

TABLE 2

Respiratory quotients during oestrous cycles

RAT NUMBER	AGE	WEIGHT	BODY SURFACE	LENGTH OF FAST	STAGE OF OESTROUS CYCLE	LENGTH OF DETERMINATION	CO_2 PRODUCED	O_2 CONSUMED	R.Q.	HEAT PRODUCTION PER DAY AT OBSERVED R.Q.*		
										Total	Per 100 grams body weight	Per square meter body surface
	days	*grams*	*sq. cm.*	*hours*		*minutes*	*grams*	*grams*		*calories*	*calories*	*calories*
4370	224	158	332	20	V	100.0	0.3828	0.3722	0.748	17.6	11.2	531
4370	228	160	335	18	Early I	311.5	1.3518	1.3236	0.743	20.1	12.5	600
4370	232	163	340	17	V	162.9	0.7113	0.7009	0.738	19.4	11.9	570
4370	237	155	328	20	Late I	200.7	0.8309	0.7994	0.756	18.3	11.8	557
4370	240	157	331	18	Late V	218.0	1.0320	0.9599	0.782	21.8	13.9	658
4370	244	165	342	18	Early I	178.5	0.9978	0.9401	0.772	21.9	13.3	640
4370	246	158	332	22	Early III	197.9	0.7361	0.7145	0.749	17.7	11.2	534
2660	238	186	370	18	Early II	79.2	0.3663	0.3716	0.717	21.9	11.8	593
2660	242	192	378	20	II	114.4	0.4824	0.4799	0.731	22.9	11.9	605
2660	245	186	370	18	Late V	77.2	0.4613	0.4620	0.726	25.7	13.8	694
2660	247	220	414	19	III	137.0	0.6281	0.6227	0.734	22.9	10.4	553
2660	247	216	409	22	IV	120.0	0.8072	0.8060	0.728	23.7	11.0	580
2660	253	194	381	18	Early I	120.0	0.9684	0.9365	0.752	24.1	12.4	633
2660	256	186	370	20	V	90.0	0.4516	0.4600	0.714	20.8	11.2	563

* Determination made only during portions of experiment in which the rat was quiet.

This formula for body surface is not altogether satisfactory, since it contains no function of body length, and since it is possible that the value of k is somewhat too high. On theoretical grounds, the metabolic rate of such a small animal as the rat should be slightly higher than that of man, per square meter of body surface. With the value of 11.36 for k, the heat production is approximately 25 Calories per square meter per hour, a value fully 30 per cent lower than that for man. However, in the present work,

since comparisons are made on the same rat at an approximately constant weight any such errors are constant and affect only the absolute results.

Respiratory quotients were determined, as described above, for all phases of the cycle in two rats. They were found to be relatively constant in rats starved 18 to 22 hours, and were not found to show consistent variations at any stage of the cycle. The results are given in table 2. The average of all respiratory quotients determined was 0.746 with a coefficient of variation of ±2.5 per cent, and in all of the rest of the determinations

TABLE 3

Basal metabolic rate determinations during the oestrous cycle

RAT NUMBER	BODY WEIGHT	HEAT PRODUCTION PER DAY PER SQUARE METER OF BODY SURFACE							
		Stage I		Stage II	Stage III	Stage IV	Stage V		Average of all stages
		First 6 hours	Remainder of period				First portion	Last 10 hours	
	grams	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*	*calories*
4371	165–175		(1) 581	(2) 545	(1) 578		(9) 527	(4) 571	546
2661	168–195	(2) 576	(3) 528	(2) 590	(4) 543	(1) 542	(10) 555	(5) 595	561
4350	165–188	(1) 700	(2) 607	(3) 623	(2) 601	(1) 574	(6) 621	(4) 710	638
518	168–176	(1) 639	(2) 536	(3) 539	(2) 536	(1) 539	(5) 515	(1) 587	540
4355	180–192	(4) 648	(2) 571	(3) 554	(4) 531	(1) 533	(6) 551	(4) 694	589
1265	174–200	(4) 608	(2) 582	(3) 567	(2) 530		(6) 552	(3) 684	586
4335	176–196	(3) 547	(4) 546	(3) 485	(3) 536	(1) 516	(5) 525	(4) 558	533
2660	186–216	(6) 681	(6) 571	(8) 593	(11) 583	(4) 595	(25) 573	(18) 665	606
4370	157–172	(6) 607	(7) 553	(9) 553	(3) 528	(5) 562	(20) 557	(9) 643	573
Number of determinations.....		27	29	36	32	14	92	52	
Mean of all determinations.....		626	560	564	557	564	558	644	580
Mean deviation..		±53	±30	±46	±33	±29	±38	±54	±55

The figures in parentheses indicate the number of observations in the stage.

this value was assumed. This figure is quite in agreement with the data published by Mitchell and Carman (1926). They found the respiratory quotient to be relatively constant in starved rats and used the value 0.75 in the most of their calculations. With the gravimetric method of carbon dioxide and oxygen determination an experiment of at least two hours' duration is essential in order to obtain an accurate respiratory quotient. The method involves weighing the animal in the cage to within one milligram, both at the beginning and end of the experiment.

The results of the determinations are presented in table 3. The heat production is expressed only per square meter of body surface, since the variation is less than when expressed per 100 grams of body weight. In

collecting the data all of the satisfactory determinations were plotted by stages of the cycle. It was found that the greater number of periods of increased metabolism were recorded within the last ten hours of stage V or the first six hours of stage I. These two stages were then arbitrarily divided at those times into two periods each. None of the other stages showed such variations within themselves. During the entire cycle except the late part of stage V and the early part of stage I, the basal metabolic rate of the rats was uniformly low. A total of 203 determinations in the nine animals was made in this portion of the cycle. The average rate was 559 Calories per square meter per day, with a coefficient of variation of ±6.7 per cent. In the last ten hours of stage V, the average of 52 determinations was 644 Calories, and in the first 6 hours of stage I, the average

TABLE 4

Percentage increase in basal metabolic rate during late dioestrum and early proestrum

RAT NUMBER	A TOTAL AVERAGE OF ALL DETERMINATIONS	B AVERAGE RATE IN LATE V AND EARLY I	C AVERAGE RATE IN ALL OF CYCLE EXCEPT LATE V AND EARLY I	DIFFERENCE B AND C	PERCENTAGE DIFFERENCE
	cals./day/sq. m.	*cals./day/sq. m.*	*cals./day/sq. m.*	*calories*	
4371	546	571	538	33	6
2661	561	589	551	38	7
4350	638	708	613	95	15
518	540	613	529	84	16
4355	589	671	548	123	22
1265	586	641	557	84	15
4335	533	554	524	30	6
2660	606	669	579	90	16
4370	573	629	554	75	14
Average....	575	625	555	72	13

of 27 determinations was 626 Calories. The average of the 79 determinations in these two periods together was 638 Calories, with a coefficient of variation of ±8.7 per cent. The total average of the 282 determinations in all stages of the cycle was 580 Calories, with a coefficient of variation of ±9.5 per cent. The conclusion would appear reasonable that the increase in metabolic rate in the late part of the dioestrum and the early part of the pro-oestrum, is a definite physiological effect. The fact that the average basal metabolic rate in late stage V is higher than in early stage I indicates that the peak of this effect is reached before the beginning of a new cycle.

All of the rats used showed the increase in metabolic rate near the end of the dioestrum, although in three the amount of the change was small. The percentage increase for each rate is shown in table 4. The average was 13 per cent of the rate during the rest of the cycle.

In figure 1 all of the determinations made on rat 2660 are plotted by stages of the cycle. More determinations were made upon this rat than upon any other. Three successive oestrous cycles, each of approximately 100 hours duration, were followed, and observations obtained for every stage. The general metabolic level of this rat was rather high but, as figure 1 shows, all of the observations are well within plus or minus 10 per cent of the smoothed curve. The increase in the basal metabolic level occurred rather suddenly, about ten hours before the end of the dioestrum. The peak of the rise occurred within seven hours, and thereafter the rate fell off through the first six hours or so of the pro-oestrum until the previous level was reached. There was no marked decrease below this level in

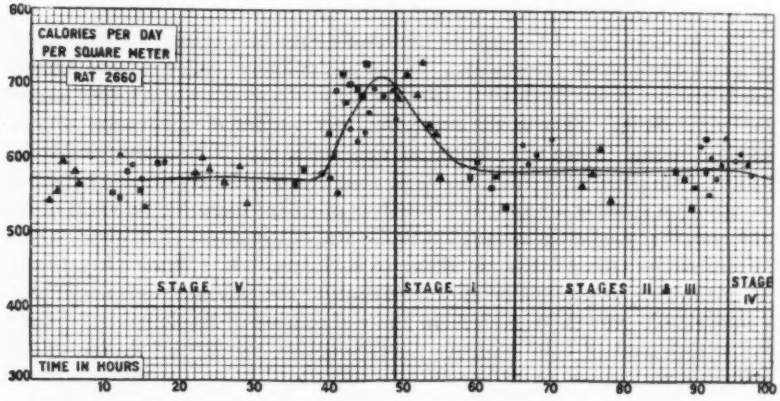

Fig. 1. Basal metabolic rate determinations in three successive cycles in rat 2660. Determinations made in the first cycle are represented by dots, those in the second by squares, and those in the third by triangles.

this rat or in any other of the series. A composite of all determinations made on all of the animals would show much the same curve. The peak would be slightly lower, and the variation somewhat greater.

There was a considerable difference in the basal metabolic level in the different rats. Three had a total average rate of less than 550 Calories, and two of more than 600 Calories per day per square meter. The coefficients of variation for the whole series are somewhat larger, therefore, than for a comparable number of observations in any individual rat. In rat 2660, for example, the total average heat production in 78 determinations was 606 Calories, with a coefficient of variation of ± 8.5 per cent. During late stage V and early I, the average of all determinations was 669 Calories, with a coefficient of variation of ± 6.3 per cent. During the remainder

of the cycle, the average of 54 determinations was 579 Calories, with a coefficient of variation of ±4.3 per cent.

Mitchell and Carman (1926) made 24 basal metabolism determinations on nine female rats and obtained the average heat production of 571 Calories per day per square meter. The total average of this series, 580 Calories, is in good agreement, despite the fact that the number of observations made late in stage V and early in stage I is much greater than would be the result of chance sampling. These times were deliberately chosen for a large number of determinations, and the high values obtained raise the total average. If the averages are weighted to give these phases only their due importance, the total average heat production in this series would be 570 Calories per day per square meter, a figure almost identical with that reported by Mitchell and Carman.

The explanation of the rise in metabolic rate just before the beginning of a new cycle is not yet evident. If the physiological cause is to be looked for in the ovary itself, there is one significant event which occurs at about the same time. This is the comparatively sudden degeneration of the corpora lutea. Since the change in metabolic rate is also comparatively sudden, the possibility suggests itself that the corpus luteum may have a metabolism sparing, or anabolic function, and that upon the removal of its influence a period of increased katabolic activity begins. The cycle in the primates offers some further evidence of such an effect. When a functional corpus luteum is present before menstruation the endometrium undergoes a remarkable growth, organization and elaboration of its elements. Upon the withdrawal of this influence shortly before menstruation, the endometrium undergoes an extensive retrogression. If no corpus luteum is present, however, the endometrium remains in the simple resting condition, even though an apparently normal catamenia occurs (Corner, 1927). The presence of a corpus luteum is probably necessary, then, for the anabolic phase of the uterine cycle. Such an explanation does not account for the rather sudden decrease of the metabolic rate to the original level in the early pro-oestrus stage in the rat. This happens nearly thirty hours before any new corpora lutea are formed.

Whatever may be the explanation, the results correlate with the findings of an increased metabolic rate just before or early during the menstrual period in women, since this period probably represents the transition between stage V and stage I in the oestrous cycle of the rat.

It is interesting to note that there was no change in the rate during stage II of the cycle, at which time the voluntary activity of the rat is from two to five times as great as at any other part of the cycle (Slonaker, 1924; Durrant, 1925). At the time of the increase in basal metabolism, on the other hand, the voluntary activity is comparatively low. Bugbee and Simond (1926) have been able to produce the increase in voluntary activity

typical of stage II by the injection of ovarian hormone into spayed rats. In this laboratory (Lee, 1928) injections of one preparation of this hormone have not shown any effects upon metabolism. It would appear that the factor which is responsible for the increase in metabolic rate is a different one from that responsible for the change in activity.

SUMMARY

A total of 282 basal metabolic rate determinations was made on nine female rats during all stages of the oestrous cycle. In all of the animals an increased basal heat production was observed in the last 10 hours of stage V (dioestrum) and in the first 6 hours of stage I (pro-oestrum). The amount of this increase above the level characteristic of the remainder of the cycle varied from 5 to 20 per cent, with an average of 13 per cent. Although this increase is not great the analysis of the data indicates that it is statistically valid. No change in the basal metabolic level was found in any other phase of the cycle. Even during stage II (oestrus) which is characterized by a marked increase in voluntary activity, the average basal heat production showed no change. The increased heat production toward the end of the dioestrum is comparable to a slight increase reported in women at menstruation.

Respiratory quotient determinations were made in two rats during all stages of the cycle. No consistent changes were found.

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QUANTITATIVE STUDIES OF THE NERVE IMPULSE

III. The Effect of Carbon Dioxide on the Action Current of Medullated Nerve[1]

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That carbon dioxide affects the magnitude of the electric response of nerve has long been recognized. Since the experiments of Grünhagen (1872) the pure gas has frequently been employed as a narcotizing agent, and Waller (1896), and later Boruttau and Fröhlich (1904), and others have noted an increase in the electric response induced by moderate concentrations. Waller employed a slow-moving galvanometer and based his conclusions on the "total action current" from a series of many impulses. Boruttau confirmed these observations, and, with Fröhlich, showed by means of the capillary electrometer that the chief element in the increase was a prolongation of the falling phase of the action current. Waller had suggested a similar interpretation. Garten (1903, 1910) found a similar effect of the gas on non-medullated nerve. None of these workers attempted to correlate the observed effects with the concentration of carbon dioxide, as it was employed merely as an agent for modifying the size or character of response.

Recently Davis and Brunswick (1926) reported a "treppe" effect in the nerve action current, and suggested the possibility that the slight increase in successive responses might be due to the accumulation of carbon dioxide. The evidence for treppe was meagre, but the effect has been confirmed by Gasser (personal communication) and Gerard (1927 b); and as carbon dioxide is a known product of nerve metabolism, although small in amount, its effects on the magnitude of response seemed worth investigation. Special interest attaches to the treppe phenomenon because of possible bearing on the question of central summation. We may state in advance, however, that the results of the present investigation do not support a theory of summation based on accumulation of carbon dioxide.

Method. For effective electrical recording it is necessary to suspend

[1] A preliminary report of these experiments was read before the American Physiological Society in April 1927. (Davis, Pascual and Rice, 1927, This Journal, lxxxi, 471.)

[2] Pensionado of the Philippine Government.

the nerve on electrodes in an atmosphere whose composition can be altered. We desired a method for rapid, and, if possible, continuous determination of the CO_2 tension in this atmosphere, and to this end arranged a closed gas circuit in which air was made to circulate by a rubber compression bulb and valves and to bubble through a solution of sodium bicarbonate of known strength. The arrangement closely resembled that of Osterhout (1918). The bicarbonate solution contained an indicator (brom thymol blue or phenol red), and from the pH thus estimated we calculated the tension of carbon dioxide in the gas phase. The method of calculation was essentially similar to that of Hastings and Sendroy (1925); (cf. also Walker, Bray and Johnston, 1927), except that we used Kendall's (1916) value for pK′, corrected for temperature and ionic strength, and solved for the CO_2 tension. The solubility coefficient for CO_2 in water was also corrected for the temperatures of our experiments.

The method gave promise of simplicity and convenience, and of fair accuracy at low CO_2 tensions, although the percentage error is considerable. Direct calibration tests with gas mixtures of known composition, checked by analysis, were most disappointing. They showed a large systematic deviation in addition to the observational error. The values calculated from the pH of the solutions were invariably high, by 80 to 100 per cent on the average. This error was independent of the partial pressure of CO_2 and of the concentration of the standard alkali (between 0.01 N and 0.0001 N), and is in the opposite direction from that anticipated from such sources as imperfect equilibration or the buffer action of the indicator itself.

We are at a loss to explain this discrepancy, but it is of interest to note that it is essentially similar in direction and order of magnitude to that found by Prof. G. H. Parker (1928) in the direct calibration of his method for determining the carbon dioxide output of nerve. Apparently this type of method for the determination of carbon dioxide requires further study before it can be considered satisfactory, and results obtained by it without direct calibration must be accepted with caution.

After the first two experiments we relied on analysis for our CO_2 tensions. Mixtures were made up under pressure in small steel cylinders, allowed to mix by diffusion for at least 24 hours, and samples taken for analysis before and after the experiment. Ordinary compressed air was employed for the controls. The gas mixture or compessed air was passed through four bubbling tubes to saturate it with moisture. The last tube contained standard alkali and indicator, and readings of pH were taken from time to time. This served to show that the water in the bubbling tubes was in equilibrium with the gas as to CO_2, and that the composition of the gas remained constant. After opening the chamber to re-moisten the nerve and when changing to a new gas mixture, the chamber was

always swept out with a relatively rapid gas current for half a minute before slowing down to the standard rate of 6 to 12 cc. per minute.

The plan of the nerve chamber is shown in figure 1. The nerve is supported by the platinum stimulating electrodes, A and B, and the eight

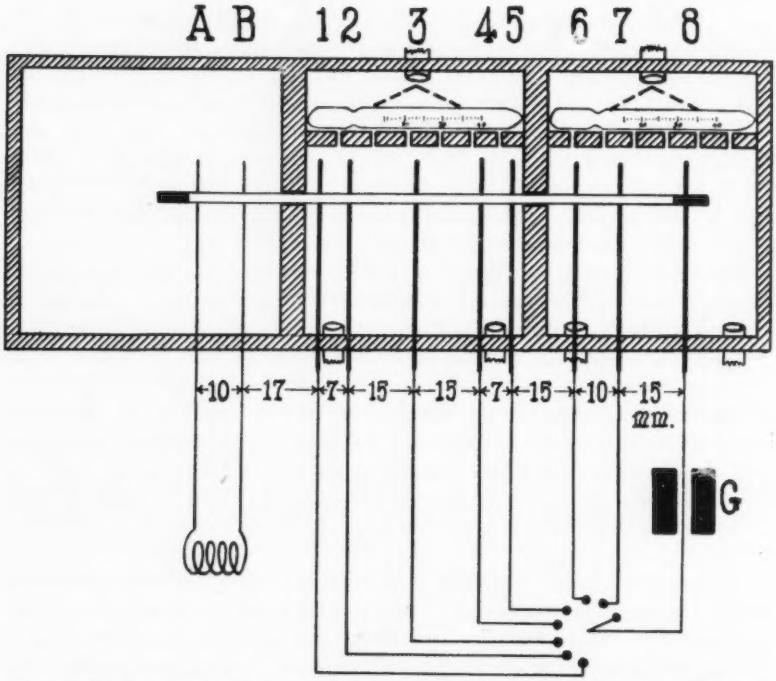

Fig. 1. Plan of gas chamber, showing positions of electrodes and distances between them. The position of stimulating electrodes A and B is varied somewhat to suit the length of the nerve. The gas enters by the tubes indicated at the top of the figure, is spread through the ante-chambers by perforated metal baffle-plates shown by the broken lines, and then passes into the nerve chambers through holes in the hard-rubber partition. These holes are at two levels, one above, one below the nerve. A small amount of distilled water is placed in the bottom of the middle and right-hand chamber and moist cotton in the left-hand chamber. Further description in text.

chloride-coated silver electrodes. It passes through slots, sealed with vaseline, in the partitions between chambers. The nerve is crushed at electrode 8, and this electrode and any one of the other seven are connected to the string galvanometer for monophasic recording.

The distances between leads are indicated, and also the position of the

gas inlets and outlets, and the diffusing chambers with their perforated metal baffle-plates. Thermometers were placed in the diffusing chambers and fair constancy of temperature ($\pm 0.3°$) was maintained by adjusting the room temperature.

The method of recording and measuring excursions of the galvanometer, of measuring the resistance of the nerve, and of calculating from these factors and the galvanometer characteristics a comparative value for the magnitude of the action current has been previously described (Davis and Brunswick, 1926). We employed a rather large string (2.5μ in diameter) under slight tension (306 m./amp.) which rendered it perfectly aperiodic. Under these conditions the height of the excursion is very nearly proportional to both the E. M. F. of a brief electrical discharge and its duration, up to 3 or 4σ. It measures quantity of electricity like a ballistic galvanometer rather than E. M. F. Our original use of "E. M. F." for the dimension of our units rested on the assumption of constant time relations in the action current, but we shall show that in the experiments to be described this assumption is unwarranted.

The nerves employed were cats' peroneals. Under ether anesthesia the sciatic was exposed from spinal column to knee, and the bundle forming the peroneal carefully dissected out and cleaned of fascia, blood vessels and outer sheath. Dissection required half to three-quarters of an hour. The nerve was washed in Ringer's solution, crushed at its central end and immediately mounted. The central end of the nerve lay on electrode 8, and in cats of average size a great enough length was obtained so that the region where the nerve begins to flatten and subdivide lay in the stimulating chamber at the left. In both of the ventilated chambers the nerve was uniform and circular in cross section and without branches.

Injury from traction was a great source of difficulty and annoyance, and could be avoided only by extreme care in manipulation. Pulling on one end of the nerve in the chamber enough to take up slack where it sagged between electrodes was sufficient in one case to reduce the action current by more than one-half in the stretched region, and although partial recovery followed it was never complete.

Local minor injuries were tested for as follows: the galvanometer was connected to leads 7 and 8 and slightly supramaximal stimuli from a balanced pair of coreless Porter coils (cf. Bishop, Erlanger and Gasser, 1926) were applied successively at A–B, 1–2, 2–3, 3–4, 4–5 and 5–6. Any increase in the response at lead 7 as the stimulus approached indicated damaged fibers which only responded at lead 7 when the point of injury did not lie between lead and stimulus. Current escape into the galvanometer, even when lead 6 served as cathode, was insignificant. By this method a point of injury could be quite sharply localized, and the test was repeated frequently during an experiment to detect possible blocking of fibers by the

carbon dioxide. Even if a few per cent of fibers were damaged originally, the experimental results are sound as long as the degree and position of the damage remained unchanged. Nerves showing a block of more than 10 per cent when first mounted were rejected. All nerves showed a slight increase (3 to 5 per cent) in response as the stimulus came nearer. Careful study, by means of our revolving mirrors, of the shape of the galvanometer excursions convinced us that this was due primarily to a more abrupt commencement of movement of the string. The peak also came definitely earlier. Apparently, in spite of the ballistic action of the slack string, the dispersion in time of individual impulses due to different conduction rates (Erlanger and Gasser, 1924) is sufficient to cause significant slowing and change of shape in the total action current. This change is readily observed when the string tension is increased to 115 meters/amp. (see fig. 2A). Therefore, the values given for "maximum degree of block," representing the increase in response at lead 7, are probably three or four per cent too high.

After mounting the nerve a slow stream of air was passed through middle and end chambers for half an hour. By this time the values for resistance, threshold and action current (as measured roughly by revolving mirrors) had become steady, and the preliminary control period was begun. Records of the electric responses to a series of single supramaximal shocks delivered at A–B were recorded from each of the seven active leads at intervals of 15 to 20 minutes, and resistance measured immediately thereafter. A rise of resistance indicates drying, and drying causes an increased action current by removing a shunt circuit. Every half-hour or so it was necessary to open the chamber, moisten the nerve and carefully drain off the excess. In this way the resistance at each lead could be kept within a range of a few thousand ohms for several hours.

After the control period the air in the middle chamber was replaced by a carbon dioxide mixture, and recording continued for at least three-quarters of an hour. Then air was readmitted for a final control period. In three experiments oxygen was substituted at the end of the control period, and in

Fig. 2A. May 4, 1927. Double exposure with revolving mirror, leads 7–8; stimulus at A–B, then 5–6. Difference in conducted distance = 72.5 mm. String tension 115 m./amp. CO_2 tension 28.0 mm. in pure O_2 in middle chamber. Pure O_2 in control chamber from which these leads are taken. Speed of film, 1 D.V. = 1/100 second. Lack of clearness in tuning fork record is due to a very slight fluctuation in the speed of the mirrors.

Fig. 2B. March 16, 1927. Leads 2–8 throughout. Shows action current remainder in the presence of CO_2 as seen with slack and with critically damped string. Speed of film constant; 1 D.V. = 1/100 second.

Fig. 2C. April 27, 1927. Lead 3, exposed to 29.2 mm. CO_2 in pure O_2 from 1:52 to 2:45. Control in pure O_2. String tension 306 m./amp. Speed of film, 1 D.V. = 1/100 second. Alternate maximal make and break shocks.

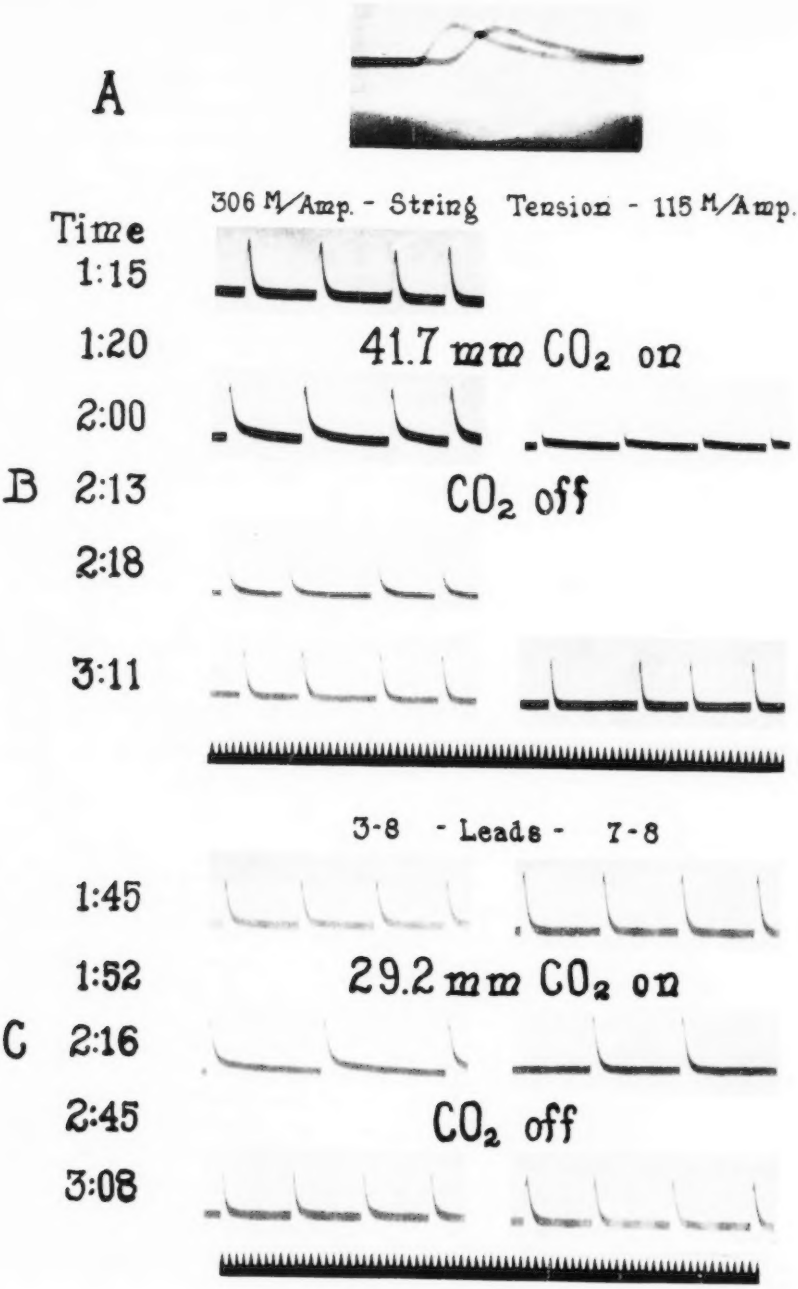
A
306 M/Amp. - String Tension - 115 M/Amp.
Time
1:15
1:20
41.7 mm CO2 on
2:00
B 2:13
CO2 off
2:18
3:11
3-8 - Leads - 7-8
1:45
1:52
29.2 mm CO2 on
C 2:16
2:45
CO2 off
3:08

Fig. 2

two cases a second carbon dioxide mixture was admitted, followed by a third control period. Throughout all experiments air was passed through the right-hand control chamber. The magnitude of response in this chamber enables us to detect possible blocking of impulses in some fibers. As long as the response here remains constant or shows only a slight progressive decay, it means that all of the fibers originally active continue to conduct, and that any diminution of response in the experimental chamber is due to a depression of response in the individual fibers and not to a total blocking of part of the fibers.

Results. Nerves kept in air under these conditions maintain a fairly constant action current at each electrode. During the first hour there is often a slight but definite increase in response. This may be due to outward diffusion of the ether used for anesthesia, but it has been seen also in nerves prepared from decerebrate animals several hours after removing the anesthetic. More probably it is due to slight drying, or to recovery from mild mechanical trauma. During the second hour the response is quite constant, and then as a rule it begins to fall off with gradually increasing speed. The behavior of control leads 6 and 7 in the experiment shown graphically in figure 3 is typical. The behavior is usually similar at all electrodes on a given nerve. It probably represents a decay or fatigue in the excised tissue, and may be due to partial asphyxia of the innermost fibers. From the formula developed by Fenn (1927) (cf. also Gerard, 1927c) we can calculate the radius of a nerve at whose center the oxygen tension is just zero when it is in equilibrium with air. We must assume that the rate of metabolism is similar to that in frog nerves at the same temperature, i.e., 0.00123 cc. O_2 per gram per minute, and that the diffusion coefficient for oxygen is the same as for muscle tissue, i.e., 1.4×10^{-5} cc. O_2 crossing an area of 1 cm. per minute under a pressure gradient of 1 atmosphere per cm. Making these assumptions we find that the critical radius is 0.95 mm. The radius of our nerves averages 0.8 ± 0.04 mm., so that probably the inner fibers were not asphyxiated, since Gerard (1927b and c) indicates that a very low oxygen concentration, 0.05 volume per cent, is sufficient for the maintenance of function of the individual fiber. In two of our experiments terminal admission of pure oxygen to the middle chamber caused a slight increase in response, but on the other hand progressive decay was evident in two experiments in which pure oxygen or oxygen plus carbon dioxide was employed instead of air.

Carbon dioxide in various concentrations had no effect on the rate of decay, for whether the gas mixture caused increase or diminution of the action current the response returned, after readmission of air, to a level very close to that which would be expected from the initial level and the rate of decay shown by the controls. We therefore consider it justifiable to interpolate a base line during the period of treatment with CO_2, as

indicated in figures 3 and 4, and to measure the increase or decrease of response relative to this.

Complete removal of carbon dioxide from the atmosphere by passing the gas over solid NaOH does not abolish or materially alter the response over a period of 3 to 4 hours. This procedure does not reduce the CO_2 tension in the nerve to zero, because the gas is constantly being produced by nerve metabolism.

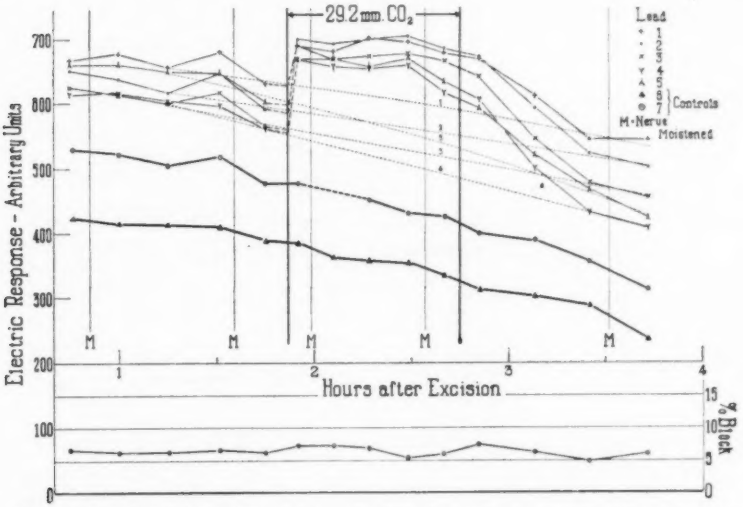

Fig. 3. April 27, 1927. Change in electric response at various points on nerve. Same experiment as figure 2C. Dotted lines are the interpolated base lines for leads, 1, 2, 3, 4 and 5 from which the percentage increases in response are calculated. The transient increase in response at 1½ hours is due to drying of the nerve. "Electric response" = (height in millimeters of peak of galvanometer excursion on film) × (Resistance of tissue in ohms + resistance of string, [18,700ω] + ρ [9,000ω,]). ρ is a factor correcting for the electromagnetic damping of the string (Davis and Brunswick, 1926). See text for method of measuring the percentage of blocked fibers. The CO_2 in this experiment was mixed with pure oxygen instead of air. The decay of response after the second hour is typical. The sharp rise of response on admission of CO_2 has been interpolated as indicated by the broken lines.

The E. M. F. of the response, as calculated, is lowest at lead 7, due to the proximity of the crushed end. At the other leads it is 50 to 100 per cent greater. As a rule they fall in the series 2, 1 and 3, 4, 5, 6, 7 in descending order, but this is not invariable and probably depends on conditions of electrical shunting. The absolute height of the galvanometer deflection, uncorrected for the different resistances introduced by the varying length of nerve in the circuit, usually shows nearly the reverse

order; 6, 7, 5, 4, 3, 2. 1. We attach importance only to *changes* in the size of response at a given lead.

Twelve experiments with tensions of CO_2 ranging from 4.5 to 250 mm. of Hg, the oxygen being constant at 150 mm., showed that up to about 55 mm. partial pressure of CO_2 the response is increased, while beyond this point it is depressed. The greatest increase is of the order of 25 to 30 per cent at tensions near 20 mm. of Hg. The depression reached 18 to 27 per cent at 250 mm. partial pressure.

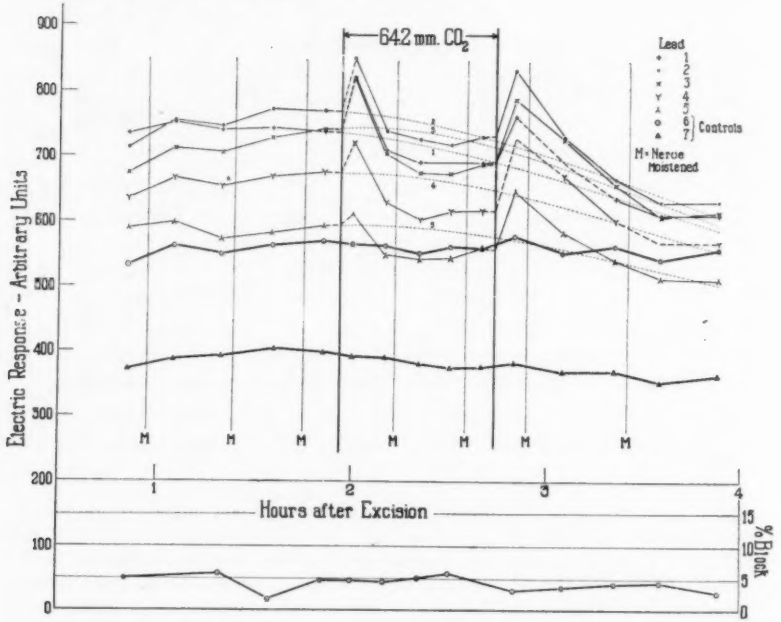

Fig. 4. March 30, 1927. See legend of figure 3. CO_2 in this experiment is mixed with air. Controls show unusually little decay. Transient rise in response at lead 4 after readmission of air has been interpolated to correspond to behavior at other leads.

The effect at various points on a given nerve is similar although differing quantitatively by as much as 10 per cent. On the whole leads 1 and 5, near the chamber walls, are affected a little less than the others. Figure 3 shows graphically the results of a typical experiment near the optimum CO_2 tension. The response increases over a period of a few minutes at each lead and then runs parallel to the interpolated base line. The per cent increase is measured as the height of this plateau above the base. The percentage of blocked fibers remains practically constant. With

TABLE 1

DATE OF EXPERIMENT	TEMPERATURE OF CHAMBER	CO_2 TENSION	MAXIMUM PERCENTAGE OF BLOCKED FIBERS	PER CENT INCREASE OF RESPONSE						REMARKS
				Lead 1	Lead 2	Lead 3	Lead 4	Lead 5	Average	
		mm. Hg	*per cent*							
December 7	23.0	[4.5]	9.0	13.0	16.0	17.0	19.0	11.0	15.0	CO_2 tension determined by indicator method, using correction factor
December 21	23.0	[10.0]	6.5	15.5	23.0	19.0	16.5	13.0	17.4	
February 3	22.9	17.7	9.0	26.0	30.9	27.2	30.7	21.2	27.2	CO_2 tension determined by analysis. Mixture contains 20 per cent oxygen
March 8	23.9	26.5	7.5	38.2	45.9	39.5	37.1	30.0	38.1	Same as February 3
March 16	24.1	41.7	8.0	5.6	6.3	5.1	6.8	7.4	6.2	Same as February 3
March 23	21.0	51.0	9.5	11.0	17.0	15.0	9.0	3.5	(11.1)	Same as February 3. Uncertainty in interpolating basal values
March 30	23.0	64.2	5.0	−5.5	−4.0	−7.5	−5.4	−7.7	−6.0	Same as February 3
April 5	22.8	93.2	7.5	−6.0	−9.0	−9.0	−10.3	−6.1	−8.1	Same as February 3
April 21	24.0	40.0	4.0	14.7	18.3	20.6	18.4	10.8	16.6	Same as February 3
April 27	23.3	29.2	6.5	14.7	23.3	27.0	27.6	18.9	22.3	CO_2 in oxygen without nitrogen
May 4	23.8	28.0	11.0	23.8	23.1	25.4			(24.1)	Same as April 27. Leads 4 and 5 discarded because of blocked fibers
May 24	23.8	[About $\frac{1}{3}$ atmosphere]	3.5	−17.9	−21.0	−24.5	−27.2	−21.9	−22.5	Gas mixture made up 33 per cent CO_2, 20 per cent O_2. Not analyzed
June 1	23.5	22.7	4.0	16.5	20.9	22.2	24.5	18.2	20.5	Same as February 3
June 20	23.1	5.4	9.0	17.2	21.0	19.8	23.8	20.6	20.5	Same as February 3
	23.1	9.7	15.0	22.7	22.3	24.3	31.9	27.6	(25.8)	Second series less reliable because of increasing block

lower tensions of CO_2 the approach to the plateau is more gradual and the return to normal more prompt. Figure 4 depicts an experiment with a higher CO_2 tension which gave ultimately a depression of response. A preliminary increase in response and a second increase on readmission of air are prominent, representing the passage of the nerve through the phase of increased response in the lower range of CO_2 tensions. There is no significant change in the percentage of blocked fibers.

In figure 5 are gathered the results of the twelve experiments correlating change of response with CO_2 tension, and two additional experiments in

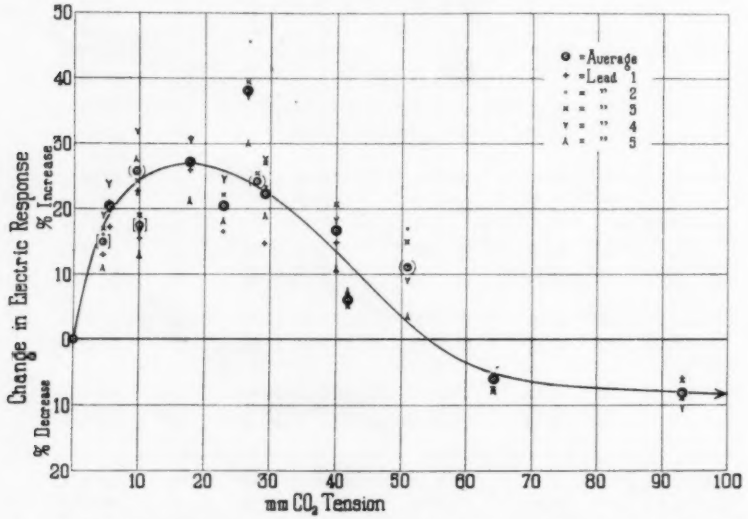

Fig. 5. Relation of per cent change in electric response of excised nerve to the partial pressure of carbon dioxide to which it is exposed. Data in table 1. The points in round brackets are less trustworthy than the others in regard to change in response; those in square brackets in regard to CO_2 tension. Experiments at 29.2 mm. and 28.0 mm. CO_2 were carried out with CO_2 in O_2 instead of CO_2 in air.

which CO_2 (partial pressure 28.0 and 29.2 mm. of Hg) was added to pure oxygen. The change at each lead, calculated from the plateau level of response, and the average for the five leads are indicated. The data include two experiments designated by square brackets, for which the CO_2 tension is somewhat uncertain, as it was determined by the indicator method and the result multiplied by an empirical correction factor, 0.55. Three other points, shown in round brackets, represent experiments less trustworthy than the remainder. Two of these, at 9.7 mm. and 28.0 mm. partial pressure, showed a blocking of fibers, which increased during exposure to the gas mixture to 15 and 11 per cent respectively. In the

experiment at 51 mm. partial pressure there is some uncertainty as to the base from which the increase should be calculated. The nerve was accidentally given very strong stimulation at all leads shortly after the readmission of air, and there is some evidence that local injury and depressed response resulted. The points shown were calculated from base lines extrapolated parallel to the base lines found for the control leads. The other nine experiments were entirely satisfactory. Another satisfactory experiment, at one-third of an atmosphere partial pressure of CO_2, is beyond the range of the figure. The average depression of response was 22.5 per cent, and the right-hand portion of the heavy line in figure 5 is drawn to pass through this point if extended.

It will be seen that the points show a general trend, which we have ventured to indicate for descriptive purposes by the curve in the figure. There is also, obviously, considerable variation from point to point on a given nerve, and some nerves, like that treated with 26.5 mm. partial pressure, seem to be atypical in the magnitude of their response. It is clear, however, that for an excised nerve there is an optimum tension between zero and the tension usually present in body tissues (45 to 50 mm.), and a depression of response above physiological tensions.

It is worth noting that these experiments further confirm the all-or-none law for nerve, as response either is affected to a similar degree at all leads or else shows nearly random variations, and not the regular sequence of depression (or increase) which a theory of decremental conduction demands. These experiments are of some additional interest, as we deal here not only with a depression but also with an augmentation of response. There is no evidence for the "transitional decrement and increment" suggested on theoretical grounds by Davis, Forbes, Brunswick and Hopkins (1926) and reported experimentally by Cattell and Edwards (1927), but we should hardly expect to detect it when using such a readily diffusible agent as carbon dioxide, although our first electrode is within 2.5 mm. of the chamber wall. True "transitional decrement" is only to be expected when the transition from normal to narcotized nerve is abrupt. Otherwise we are dealing primarily with a gradient of narcosis (cf. Kato, 1926).

During these experiments we have sought evidence of treppe in the electric response. We have used both slow (2 to 3 breaks per second) and fairly rapid rates of stimulation (20 and 30 breaks per second), but have been unable to demonstrate consistent effects of significant magnitude, either in the presence or absence of carbon dioxide. In some cases an illusion of treppe over a considerable period of time may be caused by progressive drying of the nerve. The presence of carbon dioxide makes the interpretation of a rapid series of responses difficult because of prolongation of the individual electric responses. In fact, when the string

fails to return to its original base before the next response occurs we see a diminution in the second response, although its magnitude as measured from the original base is practically unaltered. If stimulation is continued for two or three minutes the response diminishes by as much as 10 per cent, judged by any criterion, but interpretation is rendered difficult by shifts in the base, which may be due to polarization. After tetanus there may be a positive after-variation, particularly in the presence of CO_2. The base returns to its original position within a minute after the end of stimulation, and the next response is of normal size. We must leave open the question of treppe, merely noting that increase in CO_2 tension in the surrounding atmosphere does not make it more evident under our present conditions.

In the presence of carbon dioxide the electric response shows a marked prolongation. The string of the galvanometer does not return to its original base for 50 to 150σ, although normally the return is complete within 25σ or less. Figure 2, B and C, illustrates a typical "retention of action current," to use the term suggested by Levin (1927). This effect is definitely correlated with the presence of CO_2, as it is not shown by the control leads beyond the gas chamber, but occasionally we have observed it in a fresh nerve when first mounted. In such cases it gradually disappears in an hour or two. We cannot explain its appearance in these cases (cf. also Gerard and Forbes, 1928). It may be present in a nerve rapidly removed from a cat with good circulation and respiration, and absent from one taken from an animal killed by asphyxia. It usually persists longer when spontaneously present than when it has been induced by recent exposure of the nerve to carbon dioxide.

We have measured the retention of action current arbitrarily by its height above the base 30σ after the beginning of the electric response. Making allowance for the resistance in the circuit we find that at this time it may represent an E. M. F. of one millivolt, but it is usually half a millivolt or less. A study of its relation to the tension of CO_2 which evokes it shows a crude optimum at about 20 to 30 mm. partial pressure. At these tensions it is greatest both relative to the primary response and absolutely. The optimum corresponds to that for the increase in the primary response, but it must be noted that the retention of action current is found in experiments with CO_2 tensions of 65 mm. and 93 mm., under which conditions the primary response is depressed and not increased. The experiment with a CO_2 tension of one-third of an atmosphere is somewhat equivocal, since there was a distinct retention of action current present spontaneously both before and after treatment with the carbon dioxide and this was only slightly increased during treatment. Immediately after treatment, however, while the gas was diffusing out, the action current remainder was more than doubled. This indicates that there is an optimum CO_2 tension

for the remainder, and that the CO_2 effect may be superimposed upon the spontaneous manifestation.

The action current remainder dies away gradually, but the time course of the return to zero is not constant. Sometimes it is roughly logarithmic, occasionally more nearly linear. By tightening our string to the limit of periodicity at 115 meters/amp. (instead of 306 meters/amp.) we found that the retention of action current was typically not more than one millivolt at 10σ after the beginning of the response. These observations show clearly that the retention is at best small in relation to the primary disturbance, and this suggests strongly that the increase in the primary excursion is not due entirely to the appearance of the action current remainder, even though the early portion of the remainder is summated with the primary disturbance by the ballistic action of the slack string. Closer analysis awaits a more rapid recording instrument.

In the course of these experiments incidental observations were made on the threshold of excitability and on the velocity of conduction in the presence of CO_2. The threshold for both minimal and maximal response regularly rose, as measured by the resistance required in the primary of the coreless induction coils. There was sometimes a spontaneous rise during the preliminary period of equilibration or even throughout the experiment. The reversible increases associated with the carbon dioxide varied from 30 to 80 per cent when the CO_2 tension was above 20 mm. of Hg. Individual variations masked any possible correlation with CO_2 tension, but it should be noted that the threshold always rose when the nerve was exposed to carbon dioxide mixtures, whether the electric response was increased or diminished.

In three experiments measurements of velocity of conduction were carried out by means of the revolving mirrors described by Forbes and Mann (1924). This instrument causes the beam of light from the galvanometer, focussed to a narrow line, to sweep over a curved photographic film. A rotary interrupter on the same shaft as the mirrors synchronized the stimulating shocks with the movement of the mirror, so that the galvanometer excursions appear as a standing wave. To obtain exact superposition of the images for photographic recording all but one of the mirrors are hooded. The speed of the light beam over the film is 3.75 meters per second, as indicated by the standing wave of a tuning fork of 100 D. V. per second (fig. 2A). The velocity of conduction is determined by making a double exposure, recording from leads 7–8 and stimulating first at A–B, then at 5–6. The distance from B to 6 was 72.5 mm. in these experiments. The limitation on the accuracy of this method is the determination of the point on the film where the excursion begins. The string is tightened to the limit of periodicity (115 meters/amp.) to make the onset as sharp as possible, but the error of measurement may be 5 per cent. This is partly

because of the differences in conduction rate in different fibers which cause a progressive spreading of the total action potential wave as the length of nerve increases. This causes the difference in shape of the galvanometer excursions which is plainly visible in figure 2A.

By this method we were unable to detect any definite change in velocity as a result of equilibration with 28.0 mm. or 22.7 mm. partial pressure of CO_2. In the first case the velocity of the fastest impulses was about 60 meters per second before and during exposure to CO_2. In the second case it was about 50 meters per second during exposure, rose to 56 at twenty-five minutes after readmission of air, and then fell to 47 meters per second in another thirty-five minutes. This slowing is presumably to be correlated with a spontaneous falling off in the size of the electric response which took place at about the same rate. Exposure to 220 mm. partial pressure of CO_2 caused a definite slowing of conduction from 56 meters per second before treatment to 41 meters per second during exposure. The latter figure is calculated for the velocity in the treated region on the assumption that the rate remained constant in the regions outside the gas chamber. This depression of 27 per cent in velocity was associated with an average depression of the electric response of 22.5 per cent.

Discussion. Our results confirm entirely those of earlier workers. We find that a relatively high concentration of carbon dioxide depresses the electric response of nerve, which is in accord with the known narcotic action of the pure gas. It should be noted that in our experiment the oxygen tension was unchanged, so that there is no question of oxygen lack which complicates the case when pure carbon dioxide is employed. Moderate concentrations cause an increased electric response, and at least part of the increase is due to a prolongation of the state of negativity. Whether or not the rate or rise of the action potential or its maximum value are affected we cannot definitely say. The action current remainder which we observe, 10σ and more after the initial rise, is the electrical expression of the later stages of recovery. We may be dealing with an effect of carbon dioxide on the delayed portion of the recovery process which according to Gerard (1927a) is responsible for most of the heat liberated by the impulse. The relatively low potential of the action current remainder and its persistence at fairly high CO_2 tensions, when the initial excursion is reduced, indicate that it represents a step in the recovery process more or less independent of the early phases of the action current.

It is of interest that a medullated nerve exposed to CO_2 shows certain resemblances to non-medullated nerve. Levin (1927) has recently given an excellent account of electrical phenomena in crab nerve, and emphasizes particularly the "retention of action current" in the latter. The shift of the base-line of the galvanometer, causing reduction in the individual action currents, is similar to what we observe under CO_2 and our observations

support his contention that a second phase of recovery, which is responsible for the "retention," is common to all excitable tissues but is usually missed in medullated nerve because of its relatively weak intensity or brief duration.

The initial action potential wave may or may not be increased by moderate concentrations of carbon dioxide. Our observations and those of previous workers might be explained purely on the basis of a series of changes in the falling phase of the potential wave; but the simplest hypothesis is that the initial electric disturbance is increased by moderate tensions of CO_2 and diminished by higher tensions, and that some later step in the recovery process is intensified by CO_2, with an optimum at about 25 to 30 mm. partial pressure.

Nerve under the influence of a moderate tension of CO_2, showing a prolonged falling phase of the action current, an increased threshold to induction shocks, and (presumably) an increased action potential is distinctly suggestive of a nerve under anodal polarization. Our data is inadequate for comparison in other respects with the account given by Bishop and Erlanger (1926) of polarized nerve, but the parallelism is apparently not complete, for these investigators found a shortened absolute refractory phase at the anode, while Cooper's (1924) results point clearly to a prolonged absolute refractory phase under CO_2. This partial but incomplete parallelism is further evidence of the complexity of the processes involved in nerve activity and of a certain degree of independence of one another.

In any case it is of interest that the optimum tension of CO_2 for the excised nerve, as judged by the quantity of electrical flow, lies between zero and physiological tensions. Assuming this to be true for the nerve *in situ* it is apparent that an increase in CO_2 tension above normal would diminish the nerve response. The initial phases of nerve response are almost certainly depressed above 45 mm. CO_2 tension, whether or not they show an optimum at lower tensions. This is definitely opposed to the theory that summation in the central nervous system might be due to local accumulations of CO_2 and resulting treppe effects, unless we are willing to assume a much higher optimum tension of CO_2 for the synapse than for the excised nerve trunk.

Haberlandt (1911) observed that tetanization of a nerve resulted in slower conduction and that carbon dioxide caused a similar effect, and suggested that the fatigue of tetanus might be due to accumulation of carbon dioxide. Our results would support such a theory qualitatively, but a rough quantitative estimate makes it seem most improbable that this is an important factor in fatigue. The heat evolved by one impulse traversing a gram of frog nerve at 15°C. is estimated by Gerard, Hill and Zotterman (1927) as very nearly 1.0×10^{-6} cal. The R. Q. of the extra

metabolism of nerve activity is practically 1 (Gerard, 1927c) so that 1 cal. corresponds to the evolution of 0.2 cc. of CO_2. The CO_2 production of a single impulse would therefore be 0.2×10^{-6} cc. per gram of nerve. The temperature coefficient of the gaseous metabolism is about 2 (Gerard, 1927c), so that this figure becomes 0.4×10^{-6} cc. per gram at 25°C. Let us ignore entirely the buffer action of the tissue and merely consider the nerve as equivalent to 80 per cent of its volume of water. The absorption coefficient of water for CO_2 is 0.759 cc. CO_2 per cc. H_2O at 760 mm. partial pressure and 25°C. (Landholdt-Börnstein, 1923). Therefore the CO_2 produced by one impulse would increase the CO_2 tension in the tissue by

$$\frac{0.4 \times 10^{-6} \times 760}{0.759 \times 0.8} = 5 \times 10^{-4} \text{ mm. Hg.}$$

This is obviously an infinitestimal change in comparison with the alterations required to cause a perceptible difference in the response. Even a continued succession of impulses will cause no significant alteration. It may be argued that localized production of CO_2, causing differences in concentration in different regions of the fiber may cause greater changes in response, but the ready diffusibility of carbon dioxide and the small distances involved makes this seem unlikely. This does not positively exclude the possibility that in the gray matter of the central nervous system, where the metabolism is known to be more intense, the accumulation of carbon dioxide may appreciably modify response, but it makes it seem most improbable. The lowered level of electrical response in tetanus, described by Gerard (1927d) as an "equilibration," is a more likely explanation of Haberlandt's observation, and also of the reduced size of successive reflex responses (cf. Gerard and Forbes, 1928).

A point of interest in connection with the effect of carbon dioxide on nerve response is the possibility that the prolonged action current remainder may represent a persistent excitatory tendency, as Fröhlich (1909) assumes. The implications of this possibility in regard to summation effects are obvious, but without more experimental evidence speculation in this direction is futile.

We wish to thank Dr. Edwin J. Cohn for his careful criticism of our original method of measurement of carbon dioxide, Miss E. M. Bright for a number of analyses of gas mixtures, Mr. J. Emerson and Mrs. P. A. Davis for assistance in several experiments, and Miss S. Colt for assistance in preparing the paper for publication.

SUMMARY

1. The action currents from cats' excised peroneal nerves, exposed at room temperature to varying concentrations of carbon dioxide, have been recorded by means of a string galvanometer.

2. An indicator method of measuring the carbon dioxide tension in a gas mixture was tried and certain inadequacies of such a method are reported.

3. Nerves exposed to moist air and occasionally stimulated show a slow progressive diminution or "decay" in their electric responses after one or two hours. The rate of this decay is not appreciably affected by CO_2 or by O_2.

4. The initial excursion of the galvanometer, representing total current flow over a period of several sigma, shows a definite increase as the carbon dioxide tension in the atmosphere surrounding the nerve is increased to about 20 mm. At higher tensions the increase in response is progressively less, and above about 55 mm. the response is less than at very low tensions. Control observations show that the changes recorded reflect alterations in the average response of the individual fibers and not in the number of fibers active.

5. Individual nerves vary somewhat in the magnitude of the change in response. Different points on a given nerve vary over a range of about 10 per cent. The average increase in response at the optimum CO_2 tension (about 20 mm. Hg) is 20 to 30 per cent.

6. Additional evidence is obtained in support of the "all-or-none" law of nerve response.

7. The action currents of nerves exposed to carbon dioxide show a prolonged falling phase. The string may not return to its base line for a tenth of a second or more. The effective E. M. F. 10σ after the beginning of the electric response may be of the order of 1 mv. This "action current remainder" is greatest at CO_2 tensions between 20 and 50 mm.

8. At certain concentrations of carbon dioxide the primary action current is diminished while the "action current remainder" is increased. This suggests that they are the electrical expressions of different and more or less independent processes.

9. Exposure to carbon dioxide does not induce any marked "treppe" effect in successive action currents.

10. The experimental evidence does not support a theory of central summation based on the local accumulation of carbon dioxide.

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